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DIGESTIBILITY OF INDIGENOUS PLANTS UTILIZED BY *RANGIFER TARANDUS*

A

DISSERTATION

Presented to the Faculty of the

University of Alaska in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

by

Steven J. Person, B.S., M.S.

Fairbanks, Alaska

December 1975

DIGESTIBILITY OF INDIGENOUS PLANTS UTILIZED

BY RANGIFER TARANDUS

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ABSTRACT

The major goals of this study were to perfect an in vitro digestibility technique for use under field experimental conditions and secondly, to apply this technique for the evaluation of the nutritional quality of range plants consumed by reindeer and caribou. Results of the feasibility study showed that in most instances, the two stage in vitro dry matter digestibility (DMD) technique could be used for this purpose. In addition, inoculum obtained either from tamed and trained rumen fistulated reindeer or from immobilized caribou gave similar DMD values for native vegetation provided the inoculum donors had been grazing similar rangelands.

Of the mid summer forages studied, the mean DMD of grass-like plants was higher (63.8%) than forbs (60.1%), lichens (49.C%) and shrubs (43.6%). The neutral detergent fiber fraction (cellulose, hemicellulose and lignin) of the grass-like plants and lichens was higher (59.8 and 57.3%, respectively) than the forbs (31.7%) and shrubs (29.8%). The acid detergent fiber (lignin and cellulose) fraction of the vascular forage types (grass-like plants, shrubs and forbs) were all similar (24.5, 28.7 and 26.7%, respectively), whereas the acid detergent fiber content of lichens was very low (4.2%). For the vascular plants, lignin concentration in excess of 9% was the only component to show a strong correlation (inverse) with in vitro digestibility.

A possible limitation of the in vitro technique for estimating

DMD in shrubs is suggested by the finding that the in vitro digestion of shrubs was substantially less than the percentage of cell solubles components which are believed to be nearly completely digestible. Further, comparison of the in vitro and the nylon bag techniques for estimating DMD indicated lower digestibilities of shrubs and lichens with the in vitro technique than with the nylon bag technique. This suggested that the digestion of shrubs and lichens was inhibited in the closed in vitro system possibly by toxic substances or by nutrient (e.g. nitrogen) limitation.

Lastly, reindeer and caribou consume a highly varied summer diet, often in excess of 15 species of plants on a given range. Results obtained in this study indicate that the digestibility of these heterogeneous diets can be predicted by summing the product of the digestibility of individual component forages and their percentage occurrence in the diet.

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INTRODUCTION

In the field of ruminant nutrition, a knowledge of the efficiency with which foods are digested in the rumen is important for assessing the nutritional adequacy of forages. Digestibility of forage is also directly related to voluntary food intake by ruminants and has a profound influence on energy retention (Baumgardt 1970, Church 1975). For example, Blaxter (1962) has shown that an increase in digestibility of food from 50% to 60% can result in an increase in energy retention of four fold. This large multiplier effect has important implications for ecological studies. Thus the efficiency with which herbivores utilize selected plants sets limits on net productivity of individual animals and also the animal population as a whole. Hence, changes in digestibility may also influence carbon and nutrient recycling rates of harvested components of primary production. Implications of the apparent multiplier effect of digestibility on productivity has not yet been determined for arctic herbivores (White 1975).

Aided by microbial populations residing in the rumen, ruminants are able to utilize efficiently dietary cellulose and hemicellulose, structural carbohydrates that constitute a significant proportion of plant biomass. The sugars produced as end products of cellulose degradation are fermented to short chain (volatile) fatty acids, the primary source of energy for ruminants (Hungate 1966, Leng 1970). This cellulolytic activity of the rumen microbes gives ruminants a significant advantage over other herbivores as it enables them to

obtain a maximum amount of metabolizable energy from course fibrous foods. Rumen microflora also ferment other carbohydrates and hydrolyze proteins into peptides, amino acids and ammonia. The end products of protein hydrolysis may then be synthesized into other amino acids and proteins by the metabolic processes of the rumen bacteria and protozoa (Church 1975). In the rumen, therefore, ingested forage is modified into forms which can be further digested and utilized in the lower alimentary tract.

Digestion in ruminants has been investigated using a number of techniques. For example the "feeding trial", with apparent digestibility determined as the difference between nutrient intake and fecal nutrient output, has been used widely (Short 1970), however, it has the inherent disadvantage of requiring relatively large amounts of the plant species under investigation. Consequently, recent work has focused on techniques that require only small amounts of forage and make possible the simultaneous analysis of a relatively large number of plant specimens (Johnson 1966, Van Dyne 1968, Pearson 1970). These micro-digestion techniques involve the incubation of a plant sample either in the rumen itself (the nylon bag technique) or in a simulated rumen environment (the in vitro technique).

Determination of forage quality must take into account the chemical composition of the plant species, as well as its digestibility. Traditionally, the Weende system of proximate analysis has been the mainstay of animal nutritionists (Van Soest 1967). For a little over a decade, however, Van Soest and co-workers have been developing and utilizing a detergent scheme of forage analysis that measures cell

solubles and cell wall content, including cellulose, hemicellulose and lignin, parameters thought to have more biological meaning than the Weende parameters (Van Soest 1964). Although some hesitance in accepting this new system is evident, an increasing number of investigators are now using these parameters to predict digestibility of various feeds.

Since Rangifer tarandus L., reindeer and barren-ground caribou, consume heterogeneous diets, I considered that feeding trials were impractical to obtain digestibilities of the many plant types commonly utilized by these animals. Instead, the nylon bag technique and the two stage in vitro technique were used for this purpose. The detergent system of forage analysis was used in conjunction with these techniques.

One goal of this project was to determine the limitations and potentials of applying these techniques of estimating forage quality to the somewhat unique plant species consumed by Rangifer. Once these criteria were established, the digestibilities and chemical composition of a number of commonly utilized forages were determined.

The study was divided into 3 sections. The first section, Chapter I, was concerned with evaluation and modification of the in vitro digestibility technique so it could be applied to reindeer and caribou under field conditions. In Chapter II, the modified in vitro technique was used in conjunction with a nylon bag technique to evaluate the two methods of estimating digestibility and to accumulate data regarding the nutritional value of vegetation eaten by reindeer and caribou. With the knowledge and experience gained from these two studies a field study was conducted at Prudhoe Bay (Chapter III). Besides determining the

digestibility of the several plants found at this site, fiber analyses of the plants were undertaken to ascertain which factors control or limit digestibility of arctic plants. Lastly, the relative digestive efficiency of inoculum obtained from reindeer and caribou was studied to determine the feasibility of using reindeer as inoculum donors when extending the work to caribou populations.

LITERATURE CITED

Baumgardt, R. B. 1970. Control of feed intake in the regulation of energy balance. Pages 235-253. In A. T. Phillipson [Editor], Physiology of Digestion and Metabolism in the Ruminant. 636pp.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. Charles C. Thomas (Publisher), Springfield, Illinois. 332pp.

Church, D. C. 1975. Digestive Physiology and Nutrition of Ruminants. Vol. 1. Digestive Physiology. 2nd Ed. D. C. Church (Publisher), Corvallis, Oregon. 350pp.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York and London. 533pp.

Johnson, R. R. 1966. Techniques and procedures for in vitro and in vivo rumen studies. J. Animal Sci. 25(3):855-875.

Leng, R. A. 1970. Formation and production of volatile fatty acids in the rumen. Pages 406-421. In A. T. Phillipson [Editor], Physiology of Digestion and Metabolism in the Rumen. Oriel Press Ltd., Newcastle upon Tyne, England. 636pp.

Pearson, H. A. 1970. Digestibility trials: In vitro techniques. Pages 85-92. In Range and Wildlife Habitat Evaluation -- A Research Symposium. U.S. Dept. Agr., Forest Serv., Misc. Publ. 1147.

Short, H. L. 1970. Digestibility trials: In vitro techniques. Pages 79-84. In Range and Wildlife Habitat Evaluation -- A Research Symposium. U.S. Dept. Agr., Forest Serv., Misc. Publ. 1147.

Van Dyne, G. M. 1968. Measuring quantity and quality of the diet of large herbivores. Pages 54-94. In F. B. Golley and H. K. Buechner [Editors], A Practical Guide to the Study of the Productivity of Large Herbivores, IBP Handbook. Blackwell Scientific Publications, Oxford and Edinburgh. 308pp.

Van Soest, P. J. 1964. Symposium on nutrition and forage and pastures: New chemical procedures for evaluating forages. J. Animal Sci. 23(3):838-845.

Van Soest, P. J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Animal Sci. 26(1):119-128.

White, R. G. 1975. Some aspects of nutritional adaptations of arctic herbivorous mammals. Pages 239-268. In F. J. Vernberg [Editor], Physiological Adaptation to the Environment. Intext Education Publishers, New York. 576pp.

CHAPTER I.

APPLICATION OF *IN VITRO* TECHNIQUES TO ESTIMATE DIGESTIBILITY

INTRODUCTION

Determination of forage quality is an integral part of wildlife management. Of the numerous techniques developed to estimate the nutritive value of ruminant forage, the two-stage in vitro digestibility method has proved a good predictor of in vivo digestibility, especially in studies involving many forage species and mixtures of species (Oh et al. 1966; Meyer et al. 1971). The technique was originally designed by Tilley and Terry (1963) for use with forages consumed by domestic ruminants; hence, most of the information available concerning the variables inherent to in vitro digestion studies relates to relatively homogeneous diets. Recently, the technique has been applied to the wide variety of forages utilized by wild ruminants, particularly deer (e.g. Short 1971; Schwartz and Nagy 1972), elk (Ward 1971) and reindeer (den Braver 1974). Preliminary studies with reindeer showed that a number of problems were associated with the technique, principally when studying the digestibility of lichens. The technique was also unsuited for field stations not equipped with a high speed centrifuge.

This study was designed to determine the limitations of applying the in vitro digestibility technique to plant species selected by grazing reindeer and caribou. The primary objective was the determination of the effect of diet, fermentation time and treatment of inoculum on the in vitro digestibility of various forages, including lichens, when using inoculum from reindeer. Also, it had to be ascertained whether the use of a high speed centrifuge could be eliminated so

that the *in vitro* digestibility technique could be used "in the field".

MATERIALS AND METHODS

Animals and Feeding Regimens

Four adult and two yearling (number 42, 46) reindeer were used as inoculum sources; all reindeer except number 20 were rumen fistulated.

Three rations were used for this study: (1) Cattle Starter #1[a] (PCS); (2) mixed lichens (ML) consisting of 25-30% (dry weight) *Cladonia alpestris*, 45-55% *C. arbuscula*, 15-20% *C. rangiferina*, 3% *Cetraria* spp., 1% *Stereocaulon* spp. and 1% "other" and (3) mixed reindeer feed (MRDF) consisting of 67% mixed lichens (see above), 25% bromegrass (*Bromus* sp.) and 8% cured sedge (*Carex aquatilis*). Due to the limited availability of the sedge, a modified MDRF (MDRF2) containing 72.3% ML and 27.7% bromegrass was fed during a portion of the experiments. The ML, MRDF and MRDF2 were each ground in a hammer mill, mixed and stored at 0 C until fed to the animals. All animals were fed a given diet for at least 30 days prior to their use as inoculum donors.

Rumen liquor was collected from the rumen fistulated animals using the technique described earlier by Person *et al.* (1975). The apparatus consisted of a rigid plastic sampling tube (approximately 8.5 cm long, 2.5 cm in diameter, drilled with 12 holes approximately 0.5 cm in

[a]Ralston Purina Co., St. Louis, Missouri.

diameter) covered with fine nylon mesh (nylon hose). The device was connected through an Erlenmeyer flask to a 2 l syringe, which was used as a vaccuum source. Occasionally the collection device became plugged, especially when the reindeer were fed the ML or MRDF diet. Under these circumstances rumen contents (approximately 1 l) were obtained directly from the rumen, placed in a prewarmed (39 C) Dewar flask and rumen liquor was expressed from the solids through one thickness of fine nylon mesh. Reindeer number 20 was sacrificed and contents were collected from the excised rumen.

In Vitro Digestibility Technique

The two stage in vitro digestibility technique described by Tilley and Terry (1963) involves the incubation of a known weight of air dried forage with rumen liquor and a phosphate buffer (McDougal 1948). After 48 hr, the mixture is centrifuged (2000 g), the supernatent decanted, and the sediment resuspended in an HCl-pepsin solution to simulate abomasal protein digestion. After an additional 48 hr, the mixture is filtered through a sintered glass crucible leaving behind the residual dry matter. Reagent blanks are incubated concurrently to determine the amount of residual matter that originates from the inoculum. In vitro digestibility is then determined as dry matter disappearance (DMD) using the residual inoculum dry matter equation of Meyer et al. (1971):

$$\text{DMD } (\%) = \frac{\text{forage DM} + \text{residual inoculum DM} - \text{residual DM}}{\text{forage DM}} \times 100$$

Preliminary trials indicated three difficulties in using this

technique with reindeer forage: the requirement for a high speed centrifuge at remote field sites; high between-replicate variability; and difficulty in filtering and washing the substrate that remained at the termination of the experiment.

Three experiments were designed to test the requirement for a centrifuge. In the first experiment, results from control tubes (treated as above) were compared with values from samples that were directly acidified at the end of 48 hr with 2.2 N HCl to about ph 1.5 without centrifugation, using the method suggested by Tilley and Terry (1963). In the second trial the controls were compared to digestibility values in which only the first or fermentation stage was performed, similar to the "Wisconsin technique" described by Baumgardt et al. (1962). The third trial consisted of comparing the controls to samples that were frozen (-20 C for four days) between the first and second stages. This technique allowed initiation of the experiment in the field followed by transportation of the samples to a centrifuge-equipped base laboratory for centrifugation and subsequent completion of the second stage.

The high between-replicate variability and difficulties encountered in washing and filtering samples apparently are related to high and variable dry matter added to the forage with the microbial inocula. In an attempt to reduce this dry matter, the rumen liquor was centrifuged at 200 g for five minutes prior to inoculation. This supernatent is referred to as "cleared rumen liquor".

To reduce the between-sample variability in inoculum dry matter,

two methods of dispensing rumen liquor were examined. As a control, 35 ml disposable syringes were filled with rumen liquor and the first six 5 ml aliquots were expressed into centrifuge tubes containing phosphate buffer. For the other technique, an autosyringe was used to dispense rumen liquor from a 300 ml Erlenmeyer flask. Two filtering techniques were tested by filtering the contents of half of the tubes from each of these dispensing techniques through course sintered glass crucibles and half through similar crucibles containing acid washed asbestos fibers as a filter-aid.

It was anticipated that circumstances surrounding rumen liquor collection would vary from working in a warm abattoir immediately adjacent to the laboratory to collection in the field from shot specimens, with resultant potential aeration of the rumen liquor. To determine the effects of this exposure to an aerobic atmosphere, tubes were inoculated with rumen liquor that had been aerated by expressing it through an autosyringe into an open beaker every 5 minutes for 20 minutes. During this sequence, the liquor was maintained at 39 C.

Digestion Rates

To compare *in vivo* with *in vitro* digestion, the time-course of dry matter disappearance of PCS and MRDF was determined by stopping the first stage digestion at timed intervals. When the inoculum donors were being fed MRDF, 11 duplicate samples were obtained from 1 hr to 72 hr; when they were on a PCS diet, 15 duplicate samples were obtained from 0.5 hr to 96 hr. Appropriate inocula blanks were also removed from the incubation bath at the predetermined times. All samples were frozen

prior to filtration and only the first stage of the technique was used to eliminate ambiguities arising from solubilization of forage dry matter in the HCl-pepsin solution.

Solubility of forage dry matter in the buffer was determined concurrently by incubating 9 samples of PCS and MRDF separately in buffer solutions in the same time sequence as the digested samples.

Rumen Turnover Time

The standard in vitro digestibility technique uses a fixed incubation time of 48 hr, whereas in vivo, fermentation time in the rumen varies depending on the turnover time (TT) of particulate matter. Therefore, to compare directly the efficiency of the in vitro procedure with the rumen itself, in vivo results were compared to in vitro digestion when the incubation time was equal to one TT. The Ce 144 technique of Weston and Hogan (1967) was used as an indicator of particle turnover because of the affinity of cerium for food particles (Miller et al. 1967). ^{144}Ce Cl was injected through a rumen fistula and samples of mixed rumen contents were removed at regular intervals to 16 hours post-injection. Radioactivity was measured using a gamma spectrometer (ND multichannel analyzer/NaI (TL) detector) and a specific radioactivity (SA) was calculated (μ Ci ^{144}Ce/gm dry matter). The decline in SA with time following ^{144}Ce injection was plotted on semilogrithmic coordinates. The slope of the line (k, hr^{-1}) was determined by least squares regression and the TT was calculated as the inverse of the slope (i.e. 1/k, hr).

In Vivo Digestibility

Reindeer numbers 2 and 9 were confined in digestion stalls as

described by Cameron (1972) and fed the experimental diet for 4 weeks (PCS) or 5 weeks (MRDF) prior to the determination of the apparent digestibility of the feed. Feed and water intake and feces and urine output were then measured daily for 9 days. After the equilibration portion of the experiment, MRDF2 was fed instead of MRDF to conserve the limited quantity of available Carex aquatilis. In vivo digestibility was calculated as:

$$\text{in vivo digestibility} = \frac{\text{feed intake (D.W.)} - \text{fecal output (D.W.)}}{\text{feed intake (D.W.)}}$$

RESULTS

Table 1 lists the results of the experiments designed to obviate the use of a centrifuge. They indicate no difference ($P>0.05$) between direct acidification as opposed to centrifugation at the end of the first stage, but the high dry matter content of the acidified samples greatly increased filtration difficulties, especially when using the lichen Cladonia alpestris as a substrate. Thus, the acidification technique was an unacceptable alternative to centrifugation.

The mean values of the controls and the samples which had been subjected to only the first stage of the in vitro technique were not significantly different ($P>0.05$). These results were anticipated with C. alpestris due to its low protein content (<3% protein: Spencer and Krumboltz 1929). Also, since both of the techniques produced negative digestibilities of C. alpestris, these are interpreted as representing

TABLE 1. The effects of minor modifications in technique on *in vitro* dry matter disappearance.

Treatment	Animal	Diet	Dry Matter Disappearance % ± S.D. (n) Purina Cattle Starter	*Cladonia alpestris*
Centrifugation	10	PCS	75.1 ± 1.7 (3)	17.2 ± 2.1 (3)
Direct acidification	10	PCS	76.0 ± 2.0 (3)	19.1 ± 2.2 (3)
Control	42	PCS	55.0 ± 0.7 (3)	-3.9 ± 0.6 (3)
First Stage Only	42	PCS	54.3 ± 0.9 (3)	-2.0 ± 4.0 (3)
Frozen prior to 2nd Stage	42	PCS	66.9 ± 2.4[a] (3)	0.7 ± 1.8[a] (3)
Aerated rumen liquor	42	PCS	57.0 ± 2.8 (3)	0.2 ± 1.3[a] (3)
Control	42	ML	61.2 ± 1.8 (3)	22.9 ± 4.7 (2)
Cleared rumen liquor	42	ML	65.0 ± 0.5[a] (3)	18.4 ± 2.3 (3)
Control	46	ML	90.5 ± 5.7 (3)	48.1 ± 7.3 (6)
Cleared rumen liquor	46	ML	65.2 ± 0.5[a] (3)	1.2[a] (1)

[a]Mean values followed by "a" are significantly different ($P<0.05$) from the appropriate control.

zero digestibility; therefore no difference would be expected. The similar results obtained with both techniques when using PCS (ca. 11% protein) as a substrate have no logical physiological explanation, however, the high between-replicate standard deviations of both PCS and C. alpestris when subjected only to the first stage (10.9 and 4.0, respectively) compared to those of the controls (0.7 and 0.6, respectively) confirmed that the second stage protein digest tends to reduce variability, in agreement with Barnes et al. (1964).

Freezing the tubes and their entire contents at the end of the first stage resulted in significantly higher ($P<0.05$) digestibilities compared to the controls (Table 1). Freezing also caused an apparent easing of filtration, which could have accounted for the higher digestibilities due to increased washing efficiency.

Attempts to reduce inoculum dry matter by using cleared rumen fluid produced variable results (Table 1). The cleared rumen fluid from reindeer number 46 caused significantly lower results ($P<0.05$) for both PCS and C. alpestris, whereas the cleared rumen liquor from number 42 yielded significantly higher ($P<0.05$) digestibilities of PCS and slightly lower results with C. alpestris. Although the use of cleared rumen liquor resulted in considerable easing of filtration, it was discontinued because of the variable results.

In experiments designed to minimize variability in the amount of inoculum dry matter, significantly less dry matter ($P<0.05$) and lower standard deviations were obtained when rumen liquor was dispensed with an autosyringe (0.0273 ± 0.0050 g/ml) than with a 35 ml syringe

(0.0323 ± 0.0060 g/ml). Particles tended to plug the valves of the autosyringe, however, making its use undependable. Hence the shortcomings of dispensing the rumen liquor with a 35 ml syringe were accepted in subsequent experiments.

As a final attempt to increase ease of filtration, the effectiveness of asbestos as a filter-aid was tested. Acid washed asbestos fibers neither increased filtration ease nor decreased residual dry matter (0.0297 ± 0.0065 g/ml) when compared to controls with no asbestos fibers (0.0298 ± 0.0059 g/ml). Therefore, the use of asbestos was discontinued.

When whole rumen contents were collected and filtered through a nylon stocking, aeration of the inoculum was unavoidable. The results in Table 1 show, however, that the effects of aeration are minimal with a relatively rapidly digesting forage such as PCS. The significant difference ($P<0.05$) between aerated and non-aerated liquor obtained with C. alpestris as a substrate is inconclusive since near zero digestion was noted with both treatments.

Table 2 shows the between-diet, between-animal and within-animal variabilities associated with the in vitro digestion of PCS, C. alpestris and MRDF. Of the 20 possible between-diet comparisons, 8 (40%) were significantly different. Further, 67% of the PCS-ML combinations were significantly different, 38% of the PCS-MRDF and only 17% of the ML-MRDF differences were significant. Of the 45 possible between-animal comparisons, 26 (58%) were significantly different. Eighty-eight percent of the between-animal comparisons

TABLE 2. Effect of diet and animal on *in vitro* dry matter disappearance.

In Vitro Test Forage	Animal Number	Dry Matter Disappearance (% ± S.D.) PCS Diet	ML Diet	MRDF Diet
PCS	2	65.1 ± 4.9 c x	52.6 ± 3.1 b y	59.5 ± 7.6 a x, y
	2			68.9 ± 1.6 a x
	9	66.9 ± 1.7 c x	74.9 ± 5.6 a x	72.4 ± 2.4 a x
	9			67.5 ± 7.5 a x
	10	75.1 ± 1.7 b		
	20		61.3 ± 1.8 c	
	42		61.2 ± 1.8 c	
	46	55.0 ± 0.7 a x	90.5 ± 5.7 a y	
	46		57.4 ± 3.4 b, c x	
Cladonia alpestris	2		12.2 ± 4.7 b, f x	9.6 ± 2.0 a x
	9		26.5 ± 3.3 c, e x	22.0 ± 4.5 a x
	10	17.2 ± 2.1 b		
	20		9.9 ± 0.1 d, f	
	42		26.6 ± 7.2 b, e	
	46	-3.9 ± 0.6 a x	47.6 ± 3.9 a y	
	46		12.4 ± 1.6 b, d y	
MRDF	2	17.9 ± 3.3 b x		31.7 ± 1.6 a y
	2			38.3 ± 6.7 a y
	9	29.8 ± 2.9 a x		31.4 ± 0.0 a x
	9			43.7 ± 6.4 a y

Mean values within a column subgroup followed by a common letter (a-f) are not significantly different ($P<0.05$).

Mean values for each animal on a given diet followed by a common letter (x, y) are not significantly different ($P<0.05$).

on a PCS diet and 68% on a ML diet were significantly different. None of the 9 comparisons possible on the MRDF diet were significantly different. There were 6 within-animal comparisons. Those from animals on a ML diet were both significantly different but no significant differences were obtained with the four comparisons of reindeer on a MRDF diet.

The temporal change in *in vitro* DMD of PCS is triphasic using inoculum from reindeer consuming either a PCS or MRDF diet (Figure 1). The data from two reindeer were pooled to obtain this relationship since there was no significant between-animal difference ($P<0.05$) in the first and third components of the curve and only a barely significant difference ($P<0.05$) in the second.

These data show that the diet of the inoculum source animal has no significant effect ($P<0.05$) on the initial phase of PCS digestion and further, that a greater percentage of this initial phase can be accounted for by water solubility when the animals are on a MRDF diet. The second component of the curve is steeper ($P<0.05$) but shorter for the reindeer consuming MRDF. The rate of PCS digestion in the third component is also greater ($P<0.05$) with MRDF inocula, resulting in the same ($P<0.05$) PCS digestibility in 72 hrs as occurred in 96 hrs with the PCS inocula.

In vitro digestion of MRDF, instead of following three first order processes, occurs as a zero order process regardless of the source of inoculum (Figure 2). The initial lag of approximately 16 hrs was confined to inoculum from reindeer on the PCS diet. After the

FIGURE 1. Comparison of PCS digestion rates using inoculum from reindeer on a PCS diet ($y = 15.2\ e^{0.134t} + 30.0\ e^{0.016t} + 51.2\ e^{0.004t}$) and a MRDF diet ($y = 9.2\ e^{0.408t} + 19.6\ e^{0.058t} + 43.5\ e^{0.008t}$) for reindeer numbers 2 (O) and 9 (●). △ indicates dry matter disappearance (solubility) of PCS in buffer.

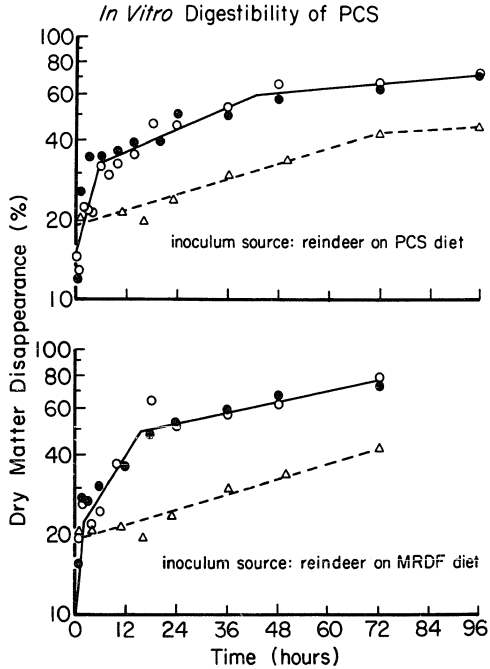
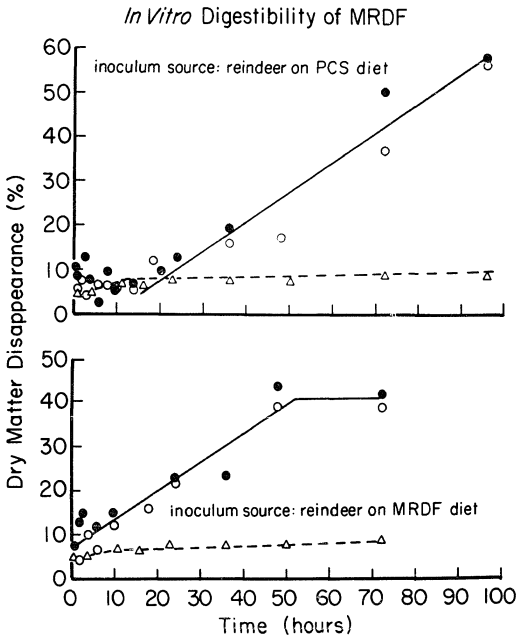


FIGURE 2. Comparison of MRDF digestion rates using inoculum from reindeer on a PCS diet ($y = 0.64x - 5.8$) and a MRDF diet ($y = 0.66x + 6.8$) for reindeer numbers 2 (O) and 9 (●). △ indicates dry matter disappearance (solubility) of MRDF in buffer.



lag phase, DMD proceeded at the same rate of 0.54%-0.65%/hr ($P<0.05$) on both diets, continued for 96 hrs with PCS inoculum, but leveled off after 48-52 hrs with MRDF inoculum.

The rumen TT listed in Table 3 indicate that reindeer given a PCS diet have a longer rumen TT (31.0, 39.2 hr) than those given MRDF (24.0, 25.2 hr). The *in vitro* digestibilities of PCS terminated at the rumen TT of the donor animal were close to *in vivo* estimates for reindeer number 9 (55.1 vs. 53.9%, respectively) but were lower for reindeer number 2 (48.9 vs. 59.0%, respectively). For MRDF, *in vitro* estimates at the rumen TT proved to be substantial underestimates of the *in vivo* values.

DISCUSSION

One shortcoming of applying the Tilley and Terry (1963) *in vitro* digestibility technique to a remote field site is the requirement of a centrifuge capable of producing 2000 g. Attempts to circumvent this requirement by utilizing only the first stage or by replacing centrifugation with direct acidification were unsuccessful. An acceptable alternative, however, was to initiate the first stage in the field and freeze the samples at the end of 48 hrs. Centrifugation and continuation of the second stage could then be accomplished in a base laboratory at a later date. Since freezing the samples enhanced ease of filtration, it was advantageous to freeze samples at the end

TABLE 3. Comparison of *in vivo* and *in vitro* digestibilities estimated at the rumen turnover time.

Forage	Animal	Rumen Turnover Time (hr)	Digestibility (%)		
			In Vivo	*In Vitro* Incubated for TT	*In Vitro* 48 hr Incubation
PCS	#2	31.0	59.0	48.4	65.1
PCS	#9	39.2	53.9	55.1	63.3
MRDF	#2	24.9	60.9	23.0	38.3
MRDF	#9	25.2	57.3	23.2	43.7

of the first stage, even when a centrifuge is available, to enable comparison of results from experiments done under varying conditions.

The high, but inconsistant between-replicate variability, was especially pronounced when using inoculum from reindeer on diets containing lichen. Bezeau (1965) found a similar dietary effect with animals on a rough fescue diet. A likely cause of this variability is the variable amount of residual dry matter (10-50% of the total) that originates from the inoculum. Attempts to reduce this variability by refining the dispensing technique or to reduce the gross amount of inoculum dry matter by slow centrifugation were not successful. Dehority et al. (1960) demonstrated that cellulolytic bacteria remain in suspension in the rumen liquor of sheep and cattle after centrifugation at 250 g, however, most large bacteria and some small bacteria associated with feed particles were sedimented. It appears that these variable effects may have caused the variable results obtained when using cleared rumen liquor as incubation medium. Based on these initial experiments, it appears that the in vitro digestibility technique can be utilized in field situations where no centrifuge is available, provided the samples are frozen to allow completion of the experiment in a base laboratory. Between-replicate variability can best be reduced by careful filtration of the rumen liquor prior to its use as inoculum, even at the expense of partial aeration of the fluid.

Several workers have investigated the effects of diet of the inoculum source animal on in vitro digestibilities. For example, Barnes (1965) emphasized the importance of the diet of the donor

animal and Short (1971) found that inoculum from deer on a laboratory ration produced higher in vitro results of that ration than inoculum from wild deer. Ward (1971) reported that the diet of elk significantly affected the in vitro digestion of grasses. Bezeau (1965) found significant dietary effects also, but the difference between inoculum donors was sometimes greater than dietary differences. Conversely, den Braver and Eriksson (1967) found that varying the ratio of hay to grain to soya meal in the diet of inoculum donors had no significant effect on the digestion of 5 different hay samples. Sources of variation other than diet have also been found. For example, Van Dyne (1962) reported significant between-animal variation and Bowden and Church (1962) and Johnson et al. (1964) reported between-day differences using the same inoculum donor on the same diet. Barnes et al. (1964) found that variation with bromegrass as a substrate was nearly twice that experienced with alfalfa.

Although the diet of the inoculum source reindeer appears to affect in vitro DMD, especially when diets are very dissimilar, e.g. PCS and ML, these dietary effects tend to be overshadowed by between-animal, and to a lesser extent, by within-animal variation. The between-animal and within-animal variabilities in this study are generally of similar magnitude to in vivo digestibility studies, e.g. with white tailed deer (Mothershead et al. 1972; Ullrey et al. 1964, 1968, 1971) or sheep (Troelsen and Hanel 1965).

Several causative factors for this variability have been identified. Kotb and Pfander (1965) reported a depressed DMD for sheep maintained

at 32.2 C compared to sheep at 5 C, possibly due to heat stress on the rumen cellulolytic bacteria. Ammann et al. (1973) found a correlation between in vitro digestibility and feed intake of white tailed deer. All rumen liquor for this study was collected from animals maintained between -35 C and +5 C, a range probably within the thermoneutral zone of reindeer. Therefore, temperature effects on the rumen microbial populations seem unlikely. Also, the reindeer were fed ad libitum so no direct correlations of digestibility with feed intake can be made.

No data is currently available concerning between-animal variations in Cervid rumen microflora, but Prins and Geelen (1971), working with red, fallow and roe deer, reported between-species and within-species differences in numbers and species distribution of rumen protozoa, even though all deer were taken from the same study area. Similarly, Dehority (1975) found a wide range in the numbers of ciliate protozoa in reindeer rumen fluid with animals on the same diet. Similar results might be expected with the ruminal bacteria populations, and therefore, could be a contributing factor to the between-animal and within-animal differences in in vitro DMD reported herein.

Hungate (1966) has described the in vitro digestion of alfalfa as occurring in three distinct phases. The first, usually very rapid and lasting less than 1 hr, is associated with the fermentation of soluble carbohydrate. The second phase, lasting from 1 to 6 hr, results from the diminished fermentation of soluble material and the initiation of fiber digestion. The third and longest phase is attributed chiefly

to fiber fermentation.

In vitro digestion of PCS appears to follow a similar pattern, however the steeper but shorter second component found with inoculum from reindeer on a MRDF diet suggests a more rapid initiation of fiber digestion by these microbes than by microbes from a PCS fed animal. This is further supported by the greater rate of PCS fiber digestion (third component) using MRDF inoculum. These observations, combined with the lag time of MRDF digestion using PCS inoculum, indicates that inocula from reindeer on a more diverse diet of higher cell wall content (MDRF) contain a more adaptable microbial population. With both substrates, however, the PCS inoculum eventually produces similar or higher digestibilities than the MRDF inoculum. Since both inoculum-source reindeer had been changed from a MRDF diet to a PCS diet only 7.5 and 9 weeks prior to being used as sources of PCS inoculum, it is likely that they retained residual populations of microbes which were capable of fermenting lichens, provided time was allowed for them to increase in abundance.

The rumen TT for animals on a PCS diet was about 3 to 4 times longer than earlier reported by this laboratory (Person et al. 1975). However, the in vivo estimates of digestibility are very similar in the two reports, which suggests that the longer residence time of food particles in the rumen, as reported herein, does not allow for a more complete digestion. Conversely, reindeer on a pure lichen diet (Person et al. 1975) had a considerably longer TT (3-5 d) than those on the MRDF diet (1 d). This reduction in TT could either be attributed to

the addition of bromegrass to the lichen, or to a more complete adaption to the MRDF diet.

In vitro digestibilities estimated at the rumen TT were substantially lower than *in vivo* MRDF digestion, and to a lesser extent, also underestimated the *in vivo* digestion of PCS in one of the two animals. The 48 hr *in vitro* estimates for PCS overestimated the *in vivo* digestibilities, but even the addition of the acid pepsin stage could not raise the *in vitro* digestibilities of MRDF enough to approach the *in vivo* results. Thus, it appears that on diets of moderate crude fiber content (e.g. PCS), long incubation times could overestimate apparent *in vivo* digestibility of the forage. On the other hand, the digestion of lichens appears to be underestimated *in vitro* and the degree of underestimation may be highly dependent on the degree of adaptation of the donor animal to lichens. Microbial adaptation *per se*, however, may not account for all of this underestimation, as the residual dry matter in the inoculum from animals consuming lichens (MRDF) was high and filtration based on this inoculum was difficult. Van Soest *et al.* (1966) indicated that some bacterial residues are retained as part of the residual dry matter, thereby reducing *in vitro* digestibility results. Further, Wilson *et al.* (1971) concluded that this effect increases in forages of low digestibility. The closer approximations of *in vivo* digestibility obtained *in vitro* with PCS than with MRDF, substantiate their findings.

It is apparent that the two stage Tilley and Terry (1963) *in vitro* digestibility technique cannot be used as a direct estimator of *in*

vivo digestion of lichens or lichen containing diets without modifications that allow for (a) a lower residual dry matter and (b) easy filtration of incubation solutions. To some extent this could be accomplished by using a lesser amount of inoculum, and perhaps a compensatory increase in incubation time as proposed by den Braver and Eriksson (1967). Even if these modifications are successful, it is unlikely that in vitro digestibility estimates will become absolute. Tilley and Terry (1963) state that in vitro digestion trials can be a guide only to the potential, rather than the realizable value of a feed. Based on the limitations and potentials in this report, it is considered that further applications of the in vitro digestibility technique to the forages of reindeer and caribou will be valuable for classifying the relative nutritive value of these forages.

LITERATURE CITED

Ammann, A. P., R. L. Cowan, C. L. Mothershead, and B. R. Baumgardt. 1973. Dry matter and energy intake in relation to digestibility in white-tailed deer. J. Wildl. Mgmt. 37(2):195-201.

Barnes, R. F. 1965. Use of in vitro rumen fermentation technique for estimating forage digestibility and intake. Agron. J. 57(2):213-216.

Barnes, R. F., G. O. Mott, L. V. Packett, and M. P. Plumlee. 1964. Comparison of in vitro rumen fermentation methods. J. Animal Sci. 23(4):1061-1065.

Baumgardt, B. R., M. W. Taylor, and J. L. Cason. 1962. Evaluation of forages in the laboratory. II. Simplified artificial rumen procedure for obtaining repeatable estimates of forage nutritive value. J. Dairy Sci. 45(1):62-68.

Bezeau, L. M. 1965. Effect of source of inoculum on digestibility of substrate in in vitro digestion trials. J. Animal Sci. 24(3):823-825.

Bowden, D. M., and D. C. Church. 1962. Artificial rumen investigations. II. Correlations between in vitro and in vivo measures of digestibility and chemical components of forages. J. Dairy Sci. 45(8):980-985.

Cameron, R. D. 1972. Water metabolism by reindeer (Rangifer tarandus). Ph.D. Thesis. Univ. of Alaska. 98pp.

Dehority, B. A. 1975. Rumen ciliate protozoa of Alaskan reindeer and caribou (Rangifer tarandus L.). Pages 241-250. In J. Luick, P. Lent, R. White, and D. Klein [Editors], Biological Sciences Publ. #18: Proc. First Int. Reindeer/Caribou Symp. Univ. of Alaska, Fairbanks, August 9-11, 1972.

Dehority, B. A., K. el-Shazly, and R. R. Johnson. 1960. Studies with the cellulolytic fraction of rumen bacteria obtained by differential centrifugation. J. Animal Sci. 19(4):1098-1109.

den Braver, E. J. 1974. Den organiska substansens smältbarhet och nedbry-tningshastighet i några renbetesväxter bestämd med VOS-metoden. (Ms).

den Braver, E. and S. Eriksson. 1967. Determination of energy in grass hay by in vitro methods. Lantbrukshögskolans Annaler. 33:751-765.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York and London. 533pp.

Johnson, R. R., B. A. Dehority, K. E. McClure, and J. L. Parsons. 1964. A comparison of in vitro fermentation and chemical solubility methods in estimating forage nutritive value. J. Animal Sci. 23(4):1124-1128.

Kotb, A. R., and W. H. Pfander. 1965. Ovine VFA metabolism under cold and hot conditions. J. Animal Sci. 24(3):892. (Abstract)

McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43(1):99-109.

Meyer, R. M., E. E. Bartley, F. Julius, and L. R. Fina. 1971. Comparison of four in vitro methods for predicting in vivo digestibility of forages. J. Animal Sci. 32(5):1030-1036.

Miller, J. K., S. C. Perry, P. T. Chandler, and R. G. Cragle. 1967. Evaluation of radiocesium as a non-absorbed reference material for determining gastrointestinal sites of nutrient absorption and excretion in cattle. J. Dairy Sci. 50(3):355-361.

Mothershead, C. L., R. L. Cowan, and A. P. Ammann. 1972. Variations in determinations of digestive capacity of the white-tailed deer. J. Wildl. Mgmt. 36(4):1052-1060.

Oh, H. K., B. R. Baumgardt, and J. M. Scholl. 1966. Evaluation of forages in the laboratory. V. Comparison of chemical analyses, solubility tests and in vitro fermentation. J. Dairy Sci. 49(7):850-855.

Person, S. J., R. G. White, and J. R. Luick. 1975. In vitro digestibility of forages utilized by Rangifer tarandus. Pages 251-256. In J. Luick, P. Lent, R. White, and D. Klein [Editors], Biological Sciences Publ. #18: Proc. First Int. Reindeer/Caribou Symp. Univ. of Alaska, Fairbanks, August 9-11, 1972.

Prins, R. A., and M. J. H. Geelen. 1971. Rumen characteristics of red deer, fallow deer, and roe deer. J. Wildl. Mgmt. 35(4):673-680.

Schwarz, C. C., and J. G. Nagy. 1972. Maintaining deer rumen fluid for in vitro digestion studies. J. Wildl. Mgmt. 36(4):1341-1343.

Short, H. L. 1971. Forage digestibility and diet of deer on southern upland range. J. Wildl. Mgmt. 35(4):698-706.

Spencer, G. C., and O. F. Krumboltz. 1929. Chemical composition of Alaska lichens. J. Assoc. Official Agr. Chem. 12(3):317-319.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. British Grassland Soc. 18(2):104-111.

Troelsen, J. E., and D. J. Hanel. 1966. Ruminant digestion in vitro as affected by inoculum donor, collection dav, and fermentation time. Can. J. Animal Sci. 46(3):149-156.

Ullrey, D. E., W. G. Youatt, H. E. Johnson, L. D. Fay, B. E. Brent, and K. E. Kemp. 1968. Digestibility of cedar and balsam fir browse for the white-tailed deer. J. Wildl. Mgmt. 32(1):162-171.

Ullrey, D. E., W. G. Youatt, H. E. Johnson, L. D. Fay, D. B. Purser, B. L. Schoepke, and W. T. Magee. 1971. Limitations of winter aspen browse for the white-tailed deer. J. Wildl. Mgmt. 35(4):732-743.

Ullrey, D. E., W. G. Youatt, H. E. Johnson, P. K. Ku, and L. D. Fay. 1964. Digestibility of cedar and aspen browse for the white-tailed deer. J. Wildl. Mgmt. 28(4):791-797.

Van Dyne, G. M. 1962. Micro methods for nutritive value evaluation of range forage. J. Range Mgmt. 15(6):303-314.

Van Soest, P. J., R. H. Wine, and L. A. Moore. 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. Pages 438-441. In Proc. X Intern. Grassland Congr., Helsinki, Finland, Paper 20.

Ward, L. A. 1971. In vitro digestibility of elk winter forage in southern Wyoming. J. Wildl. Mgmt. 35(4):681-688.

Weston, R. H., and J. P. Hogan. 1967. The digestion of chopped and ground roughages by sheep. I. The movement of digesta through the stomach. Aust. J. Agric. Res. 18:789-801.

Wilson, A. D., W. C. Weir, and D. T. Torell. 1971. Comparison of methods of estimating the digestibility of range forage and browse. J. Animal Sci. 32(5):1046-1050.

CHAPTER II.

ESTIMATES OBTAINED USING *IN VITRO* AND NYLON BAG TECHNIQUES

INTRODUCTION

In the previous chapter some boundry conditions were determined regarding the use of the *in vitro* digestibility technique as a method for studying *Rangifer tarandus* forage. These initial studies were prompted by the requirement to obtain an estimate of the availability to reindeer and caribou of dry matter, energy and nutrients in the diet. Reindeer and barren-ground caribou inhabit tundra ranges and apparently consume a highly heterogeneous diet. In summer, they consume grasses, sedges, lichens, fungi and shrubs, including the leaves of willows and birches. In winter their diet may consist of up to 50% perennial plants and shrubs, but generally lichens are dominant (Kelsall 1968). Limited data are available concerning the nutritional value of these forage species in North America. Kennedy and Titus (*ca.* 1932), and Palmer (1944) studied *in vivo* digestibilities of various lichen mixtures, most of them unidentified as to species. Nordfelt *et al.* (1961) and den Braver (1974) plus a review by Presthegge (1954) report digestibilities of a number of species of forage utilized by reindeer in Scandinavia and Aksenova (1937) reported on the digestion of forages found in Russian Siberia. Courtright (1959) summarized earlier works on the chemical composition of many summer forages, as well as several lichens, and Scotter (1965, 1972) and Pegau (1968) have made more recent studies on the chemical composition of a number of plant species important to reindeer and caribou. Digestibility *per se* however, has not been reported for summer forages or for individual lichen species on this continent.

This study reports on estimates of digestibility of dietary components using two microtechniques: the *in vitro* digestibility technique as proposed by Tilley and Terry (1963) and modified by Person *et al.* (1975 and Chapter I) and the "nylon bag" technique in which plant samples are suspended in nylon bags within the rumen (Van Keuren and Heinemann 1962). *In vitro* and nylon bag estimates of digestibility were made with a number of shrubs, grasses, sedges, mosses and lichens that are known to be utilized as forage by both reindeer and caribou.

MATERIALS AND METHODS

Animals and Feeding Regimes

The two adult reindeer used in this study were a rumen fistulated bull (No. 2) and cow (No. 9). To provide as versatile a rumen micro-flora and fauna as possible, the animals were gradually changed from a Cattle Starter #1 (PCS)[a] diet to a "mixed reindeer feed" (MRDF) diet which consisted of 67% lichens [25-30% (dry weight) *Cladonia alpestris*, 45-55% *C. arbuscula*, 15-20% *C. rangiferina*, 3% *Cetraria* spp., 1% *Stereocaulon* spp., and 1% "other"], 25% Bromegrass (*Bromus* sp.) and 8% cured sedge (*Carex aquatilis*). All components were ground in a hammer mill, thoroughly mixed, and stored at 0 C until fed to the reindeer. After a 2 week "changeover" period, the reindeer were fed MRDF *ad libitum*

[a]Ralston Purina Company, St. Louis, Mo.

for 2 weeks prior to the first experiment. Both animals were maintained at 5 C at ambient photoperiod.

Forages

Table 1 lists the plants and plant parts that were studied. All plant samples except 5 lichens were collected near Nome, Alaska in September, 1972. Three lichen species were collected on the Kenai Peninsula and 2 others were collected near Cantwell, Alaska. All samples were ground through a 20 mesh screen in a Wiley Mill prior to being used as substrates.

A mixture of several of the species was used in all trials as an indicator of between-animal and between-day variation. This "standard reindeer forage" (SRF) was composed of 40% (dry weight) lichens (20% Stereocaulon alpinum, 10% Cetraria islandica, 10% Cladonia arbuscula), 20% grass (Festuca altaica), 25% sedge (15% Eriophorum vaginatum and 10% Carex aquatilis), 10% shrub (Salix pulchra) and 5% moss (Sphagnum magellanicum).

To facilitate comparison of plant species from different trials and using different digestors, all dry matter disappearance data were adjusted by multiplying by a conversion factor, which is the quotient of the mean SRF digestibility obtained from all nylon bag or in vitro trials divided by the SRF digestibility for each specific trial.

Determination of In Vitro Digestibility

In vitro digestibility was determined using the method of Tilley and Terry (1963) as modified by Person et al. (1975). Briefly, this consisted of a 48 hr incubation in rumen liquor and a phosphate buffer

TABLE 1. Plant species and parts used for dry matter digestion trials in reindeer, collected September 1972, near Nome unless otherwise noted.*

Species	Habitat	Parts Used
Lichens		
Cladonia alpestris - Nome	Dwarf shrub-lichen	Living podetium
Cladonia alpestris - Kenai	Dwarf shrub-lichen	Living podetium
Cladonia alpestris - Cantwell	Dwarf shrub-lichen	Living podetium
C. arbuscula	Dwarf shrub-lichen	Living podetium
C. rangiferina	Dwarf shrub-lichen	Living podetium except as otherwise noted
C. uncialis	Dwarf shrub-lichen	Living podetium except as otherwise noted
Cetraria islandica	Dwarf shrub-lichen	Entire thallus
C. culcullata - Nome	Alpine dryas	Entire thallus
C. culcullata - Cantwell	Dwarf shrub-lichen	Entire thallus
Alectoria nigricans	Alpine dryas	Entire thallus
Thamnolia vermicularis	Alpine dryas	Entire thallus
Stereocaulon alpinum	Exposed gravel beds	Pseudopodentia and cephalodia
S. rivulorim - Kenai	Exposed gravel beds	Pseudopodentia and cephalodia
Peltigera aphthosa	Dwarf shrub-lichen	Living lobes
Lobaria linita	Dwarf shrub-lichen	Living lobes

TABLE 1. Continued.

Species	Habitat
Mosses	
Hylocomium splendens	Dwarf shrub-lichen
Polytrichum juniperinum	Dwarf shrub-lichen
Sphagnum magellanicum	Wet bog
Grass-like Plants	
Sedges	
Carex aquatilis	Periphery of ponds
C. lyngbyaei	Periphery of ponds
C. bigelowii	Dwarf shrub-lichen
Eriophorum vaginatum	Sedge tussock
E. angustifolium	Sedge meadow

Parts Used
Almost entirely gametophytes
Almost entirely gametophytes
Almost entirely gametophytes
As noted; mature but still mostly green
As noted; mature - no green material
Culms, spikes and mostly leaves; mature with some green material
Culms, spikes and mostly leaves; mature but still mostly green
Culms, spikes and mostly leaves; mature, no green material

TABLE 1. Continued.

Species	Habitat	Parts Used
Grasses		
Festuca altaica	Dwarf shrub-lichen	Culms, spikes and mostly leaves; mature, no green material
Hierochloe alpina	Dwarf shrub-lichen	Culms, spikes and mostly leaves; mature, no green material
Calamagrostis canadensis	Dwarf shrub-lichen	Culms, spikes and mostly leaves; mature, no green material
Brome hay	Cultivated fields	Green bales
Shrubs		
Salix pulchra	Willow stands	Mature leaves, some green material
Betula nana	Dwarf shrub-lichen	Mature leaves, no green material
Vaccinium vitis-idaea	Dwarf shrub-lichen	Green leaves and attached stems
Ledum decumbens	Dwarf shrub-lichen	Green leaves and attached stems
Loiseleuria procumbens	Alpine dryas	Green leaves and attached stems
Dryas octopetala	Alpine dryas	Green leaves and attached stems

*Table taken from Pegau, personal communication and Pegau *et al.* (1973).

(McDougall 1948). Care was taken to keep the liquor anaerobic and the dry matter content of the liquor was minimized by careful straining through nylon hose (see Chapter I). After the 48 hr incubation the entire solution was frozen and when convenient, was thawed, centrifuged, the supernatent was decanted and the sediment was incubated for 48 hr with an HCl-pepsin solution. The solution was then filtered, washed and the undigested particles were dried to determine dry matter disappearance (DMD).

Determination of Nylon Bag Digestibility

Nylon bag digestibilities were obtained using modifications of the technique described by Van Keuren and Heinemann (1962) and Pegau and Bos (1972). Approximately 1 g of forage was sealed into each tared 3 cm x 7 cm bag of parachute nylon. Twenty-two to 27 bags were then connected to a piece of perforated flexible plastic tubing and placed into the rumen through a one inch cannula. Several bags in each group contained steel ball bearings as weights, allowing constant mixing with the rumen contents, neither floating to the top nor sinking to the bottom. After 48 hr in the rumen the bags were removed, washed to remove particles clinging to the outside, dried at 100 C, and weighed. Dry matter disappearance was calculated as per cent disappearance of forage from the bag.

RESULTS

In vitro and nylon bag digestibilities from both animals in all trials were standarized using the conversion factors calculated from the mean SRF digestion (Table 2). Since there were significant differences ($P<0.05$) in SRF digestion between animals and trials, these conversion factors were calculated to enable direct comparison of all forages listed, regardless of the trial or animals used as a digestor. The mean nylon bag digestion of SRF (48.8%) was significantly higher ($P<0.05$) than the mean *in vitro* digestion (30.2%). No between-technique correction was made for this difference. For the forages that were tested in both animals, the use of these correction factors produced significantly lower ($P<0.05$) between-animal differences than the unadjusted results obtained using the *in vitro* technique, but only slightly lower differences with the nylon bag technique.

To test the hypothesis that the digestibility of the mixed diet is equal to the summation of the component forages, the digestion estimates for MRDF and SRF were compared with the sum of the digestibilities of their component forages (Table 3). The calculated values of 30.6% and 26.6% *in vitro* digestion of SRF and MRDF, respectively, compare well with the experimentally determined values of 30.2% and 26.5%, respectively. The summed component values of 44.3% and 47.5% nylon bag digestibility of SRF and MRDF are also close to the experimentally obtained values of 48.8% and 44.8%, respectively, although not as close as those obtained *in vitro*.

TABLE 2. Digestibility of Standard Reindeer Forage using both nylon bag and *in vitro* digestion techniques.

Animal	Trial #	Observed DMD	Adjusted DMD	Conversion Factor
9	1i	26.8 ± 2.8	30.2	1.13
2	1i	21.1 ± 0.3	30.2	1.43
9	2i	40.5 ± 6.1	30.2	0.75
2	2i	32.5 ± 0.7	30.2	0.93
9	1n	43.0 ± 2.5	48.8	1.13
2	1n	55.5 ± 1.8	48.8	0.88
9	2n	45.4 ± 3.0	48.8	1.07
2	2n	50.1 ± 1.2	48.8	0.97
2	3n	48.2 ± 2.1	48.8	1.01
9	4n	36.1 ± 0.9	48.8	1.35
2	4n	48.2 ± 0.4	48.8	1.01
9	5n	45.5 ± 0.6	48.8	1.07
2	5n	54.5 ± 2.3	48.8	0.90
9	6n	51.9 ± 1.6	48.8	0.94
2	6n	57.9 ± 4.7	48.8	0.84

i = *in vitro*

n = nylon bag

TABLE 3. Calculation of component digestibilities of Standard Reindeer Forage and Mixed Reindeer Feed. Inoculum was obtained from reindeer consuming the MRDF diet (see text).

Species	% Comp.	In Vitro Dig.	Contribution to Mixed Dietary DMD %	Nylon Bag	Contribution to Mixed Dietary DMD %
Standard Reindeer Forage (SRF)					
Cetraria islandica	10	28.6	2.86	61.6	6.16
Cladonia arbuscula	10	9.6	0.96	48.2	4.82
Stereocaulon alpinum	20	13.9	2.78	39.5	7.90
Festuca altaica	20	53.0	10.60	43.4	8.68
Eriophorum vaginatum	15	35.2	5.28	31.0	4.65
Carex aquatilis	10	59.0*	5.90	53.1	5.31
Salix pulchra	10	20.1	2.01	66.5	6.65
Sphagnum magellanicum	5	4.4	0.22	3.4	0.17
Predicted DMD of SRF			30.6		44.3
Determined DMD of SRF			30.2		48.8
Mixed Reindeer Feed (MRDF)					
Lichen mixture	67	19.9	13.13	42.5	28.47
Brome hay	25	46.0	11.50	61.1	15.27
Carex aquatilis	8	24.6	1.97	47.5	3.80
Predicted DMD of MRDF			26.6		47.5
Determined DMD of MRDF			26.5		44.8

*Estimated as 12% higher than the nylon bag estimate (53.1) based on the observation that the in vitro digestibility of comparable grass-like plants average 12% higher than the nylon bag digestibility.

The digestibilities obtained with both the in vitro and the nylon bag techniques are presented in Table 4. All of the data were corrected by multiplying the experimental estimate by the appropriate conversion factor from Table 2.

All three species of mosses tested (Table 4) had very low digestibility. Both techniques yielded similar results for Polytrichum juniperinum and Sphagnum magellanicum but the in vitro digestion of Hylocomium splendens, though only 16.1%, was significantly higher ($P<0.05$) than the nylon bag result.

The adjusted dry matter digestibility of lichens was highly variable between species. In vitro estimates ranged from 9.5 to 82.4% and the nylon bag estimates from 20.5 to 94.8%, however, all of the in vitro digestibility estimates were less than the comparable nylon bag estimates. The between-technique differences ranged from 1.8 to 52.9 digestibility units with a mean difference of 21.4 units. With both techniques Cetraria spp., except C. nivalis, were highly digestible, as were Thamnolia vermicularis and Alectoria nigricans.

Significant ($P<0.05$) differences due to geographic location were found between Cladonia alpestris from Nome and the Kenai Peninsula using the nylon bag technique. There was also a large difference in the in vitro digestibility of C. alpestris obtained from Nome compared with that from Cantwell, although it was not statistically significant due to the high standard deviations. A significantly higher ($P<0.05$) nylon bag digestibility was found in the living portions of C. rangiferina collected at Cabin Rock 1 than any other location except

TABLE 4. Nylon bag and in vitro digestibilities of some representative reindeer and caribou forages. Inoculum was obtained from reindeer consuming the MRDF diet (see text).

Forage	Trial	Adjusted In Vitro	S.D.	Trial	Adjusted Nylon Bag	S.D.
Purina Cattle Starter	1i, 2i	67.4[a,b]	15.00	2n	71.9[a]	5.9
Mixed Reindeer Feed	2i	26.5[a]	4.2	1n,2n,6n	44.8[a,b]	7.9
Mixed Reindeer Feed Components						
Lichens	2i	19.9[a]	8.4	2n	42.5[a]	4.5
Carex aquatilis	2i	24.6[a]	9.5	2n	47.5[a]	4.5
Bromegrass	2i	46.0[a]	3.3	2n	61.1[a]	4.6
Mosses						
Hylocomium splendens	1i	16.1[a]	5.4	3n	5.9	2.3
Polytrichum juniperinum	1i	13.6[a]	1.7	4n	13.2	0.4
Sphagnum magellanicum	2i	4.4	3.0	5n	3.4	1.7
Lichens						
Alectoria nigricans	2i	41.9	4.0	5n	94.8	4.1
Cetraria cucullata - Nome	1i	78.5[a]	2.9	3n	90.0	3.4
Cetraria cucullata - Cantwell	1i, 2i	82.4[a,b]	6.1			
Cetraria islandica	2i	28.6	0.6	5n	61.6	2.5
Cladonia alpestris - Nome	2i	9.5	2.0	4n	52.8	1.6
Cladonia alpestris - Kenai				4n	42.8	2.3
Cladonia alpestris - Cantwell	1i, 2i	18.2[a,b]	8.7			
Cladonia arbuscula	2i	9.6	0.4	1n	48.2[a]	22.9

TABLE 4. Continued.

Forage	Trial	Adjusted In Vitro	S.D.	Trial	Adjusted Nylon Bag	S.D.
Cladonia arbuscula - rangiferina (mixture)				2n	51.1[a]	2.8
Cladonia rangiferina						
First stage of growth				5n	52.3	2.1
Entire podetia	2i	37.4	1.0	5n	40.9	2.5
Decadent portion	2i	18.1	1.8	5n	20.5	0.6
Live portions						
From Beltz				5n	42.9	6.8
From Snake				5n	39.6	4.7
From Cabin Rock 1				5n	54.8	5.3
From Cabin Rock 2	2i	24.1	9.8	5n	42.2	3.8
Cladonia uncialis	2i	33.4	14.1	5n	35.3	2.5
Lobaria linita	2i	33.7	2.8	4n	46.4	1.1
Peltigera aphthosa	2i	40.6	1.9	1n	49.2	6.5
Stereocaulon alpinum	2i	13.9	0.3	1n	39.5[a]	13.8
Stereocaulon rivulorium - Kenai				4n	44.3	2.1
Thamnolia vermicularis	2i	43.9	3.1	4n	70.9	7.2
Grass-like						
Calamagrostis canadensis				4n	36.2	1.3
Carex aquatilis						
Green leaves				6n	53.1	2.2
Dead leaves				6n	31.7	1.4
Culms				6n	35.5	1.5
Bases				6n	26.5	3.1
Inflorescences				6n	43.2	3.1

TABLE 4. Continued.

Forage	Trial	Adjusted In Vitro	S.D.	Trial	Adjusted Nylon Bag	S.D.
Carex bigelowii	1i	40.0[a]	1.7	3n	32.8	0.8
Carex lyngbyaei						
Green leaves	1i	73.1[a]	4.7	6n	56.7	2.6
Dead leaves				6n	48.8	3.7
Culms				6n	53.3	3.9
Inflorescences				6n	48.0	2.2
Eriophorum angustifolium	1i	40.5[a]	4.2	4n	35.3	3.7
Eriophorum vaginatum	1i	35.2[a]	2.8	1n	31.0[a]	4.0
Festuca altaica	1i	53.0[a]	5.4	3n	43.4	2.5
Hierochloe alpina	2i	56.1	3.2	4n	63.7	7.8
Shrubs						
Betula nana	1i	30.9[a]	8.8	3	57.2	1.9
Dryas octopetala	2i	11.6	6.0	4	54.2	2.5
Ledum decumbens	1i	18.5[a]	1.3	5	47.6	4.2
Loiseleuria procumbens	2i	12.4	0.9	5	58.2	1.3
Salix pulchra	2i	20.1[a]	1.5	1	66.5[a]	9.5
Vaccinium vitis-idaea	1i	19.8[a]	4.4	4	64.4	1.3

a = mean of two animals.

b = mean of more than one trial.

Beltz. This higher digestibility may be related to its location on a windswept, heavily grazed ridge, facilitating the collection of recent growth specimens. Of the three stages of growth of that plant, the young podetia were the most digestible ($P<0.05$), followed by the entire podetia, with the decadent portion being significantly lower ($P<0.05$) than the other stages.

In contrast to the lichens, the *in vitro* digestibility of all the grass-like plants except *Hierochloe alpina* was higher than comparable nylon bag data. The green *Carex lyngbyaei* leaves were the most digestible of the grass-like species *in vitro* and were second only to *Hierochloe alpina* when using nylon bags. Although the green leaves and inflorescences of *C. lyngbyaei* were slightly more digestible than those of *C. aquatalis*, the dead leaves and culms of the former were significantly higher ($P<0.05$) than the latter. Since reindeer and caribou seek out the green leaves in preference to the other portions (Kelsall 1968), these two sedges are probably of equivalent value to these animals, followed by *Hierochloe alpina*, *Festuca altaica*, *Calamagrostis canadensis*, *Eriophorum angustifolium*, *Carex bigelowii* and *E. vaginatum* in descending order of digestibility.

The *in vitro* digestibility of all six shrub species was significantly ($P<0.05$) lower than values obtained using nylon bags. Based on the *in vitro* data, shrub digestion was lower than anticipated, with a range of 11.6 to 30.9%. With the nylon bag technique, however, a range of 47.6 to 66.5% DMD was obtained.

DISCUSSION

It was anticipated that the in vitro and the nylon bag techniques would approximate each other, or at least parallel each other across all of the plant groups. The responses obtained, however, were strikingly group specific, with the in vitro results being much lower than the nylon bag digestibilities of all of the lichens and shrubs, and slightly higher in 5 of the 6 grass-like plants examined. The higher in vitro digestibilities in these monocotyledonous plants is not unexpected, since there is no enzymatic protein digestion stage in the nylon bag technique, and the monocotyledonous plants are high in protein (9.9 to 27.5% DM: Igoshina 1937).

The lichens and shrubs investigated, with the possible exception of the shrub Loiseleuria procumbens (Banfield 1954) are all of medium to high palatability for reindeer and caribou (Banfield 1954; Kelsall 1968). Since these species make an important contribution to the Rangifer diet, it seems unlikely that they are as indigestible as indicated by the in vitro results. Therefore nylon bag data may provide a better estimate of the nutritive value of these forages.

The potential causes of variability inherent to the in vitro technique have been well documented, and include sample preparation, diet and species of inoculum donor, sample size, time of day of inoculum collection, between-day and between-animal variability, among others. None of these are applicable to the present experiments however, since the same two reindeer on the same dietary regime were

used for both techniques. Further, there was no between-trial trend evident with either technique. Therefore the differential results obtained with the two techniques are probably related to the fact that the in vitro technique is a "closed system" as opposed to the "open flow" system of the nylon bags suspended in the rumen. Firm conclusions regarding these differences cannot be made without further study, but two possible causes for the low in vitro estimates may be inhibition by toxic substances or inhibition due to limited nitrogen availability.

1. Inhibition by toxic substances: Nagy et al. (1964) have reported a broad spectrum of antibacterial properties in essential oils extracted from sage brush and Oh et al. (1967, 1968) found that essential oils from vinegar weed, California Bay, rosemary, California mugwort, blue-gum eucalyptus, sage brush, Douglas fire and Jerusalem oak exhibited varying degrees of inhibition on in vitro digestion by both sheep and deer using total gas and VFA production as indicators of digestion. Although no attempt was made to isolate such inhibitory compounds, their presence can be anticipated in both the lichens and the shrubs. Considerable information is available concerning the antibacterial properties of lichens, primarily attributable to "lichen acids" (see Ahmadjian 1967). Usnic acid, for example, is extracted from various Cladonias and used as a topical antibiotic in Russia and the Scandinavian countries. Burkholder et al. (1944), Burkholder and Evans (1945), Vartia (1949) and Bustinza (1951) among others have reported strong antibacterial properties of many lichens, including several commonly regarded as prime reindeer and caribou forage. Hale (1961, 1967) estimates that

approximately half of the lichens found in temperate regions inhibit bacterial growth. Evergreen shrubs, such as Dryas, Ledum, Loisleuria and Vaccinium often contain alkaloids or terpenes, similar to the monoterpene alcohols that have been shown to inhibit in vitro systems (Oh et al. 1968). The presence of toxic compounds and the fact that reindeer consume various amounts of these forages is not necessarily contradictory: Freeland and Janzen (1974) have stated that a rumen can function on a diet of up to 50% plants containing high concentrations of essential oils and phenols -- presumably through adaptation of rumen microflora. If such toxic substances are present in either the lichens or shrubs, their effectiveness would be much reduced by dilution and removal from the "open flow" rumen, thus causing the differential effects found between the in vitro and nylon bags techniques. However, in vivo digestibility of the shrubs may be less than the DMD estimated from the nylon bags as they were suspended in the rumen for 48 hr, whereas the residence time in vivo would be less (10-25 hrs, Chapter I).

2. Limited nitrogen availability: Lichens are generally very low in nitrogen, often containing less than 3% protein (Spencer and Krumbotz 1929). Likewise, by fall when the shrubs for this study were collected, they have likely translocated most of the protein from the leaves. Although many grass-like species translocate some protein to the roots in the fall, a significant proportion is usually stored in the base of the stems, which was included in these samples, so we suspect that the N content of the monocots was definitely higher

than the lichens, and possibly higher than in the shrubs. Since no nitrogen containing compounds were added to the inoculum-buffer-substrate mixture during the incubation, the demands placed on the limited supply of available nitrogen by rapidly reproducing microbes could cause rapid depletion of available N and fermentation in vitro may decline due to nutrient limitation. In the rumen, however, nitrogen concentration is maintained by recycling processes, hence fermentation capacity may be maintained and DMD from nylon bags may be optimal. Again, the duration that nylon bags were suspended (48 hrs) may be longer than the particle residence time of lichens in a lichen based diet (25 hrs, Chapter I) although lichen residence times as long as 3-5 d have been reported for reindeer during adaptation to lichens (Person et al. 1975).

Although evidence is sound for N limitation in in vitro systems, the evidence is tenuous for the shrubs. The most plausible explanation of the results in these studies may be that in vitro digestibility estimates of shrubs are underestimates due to the buildup of toxic products and that the in vitro digestibilities of lichens are underestimates due to the buildup of toxic products as well as the limitation of N and/or other nutrients. On the other hand, DMD estimates using nylon bags may be overestimated because they may be suspended in the rumen too long. In spite of this limitation, the ranking of digestibilities estimated with the nylon bag techniques are probably valid. Clearly, further investigations are necessary to determine whether only one or a combination of both of these is the causative factor for the low in vitro digestibilities of lichens and shrubs relative to the nylon bag results.

It must be emphasized that the digestibilities in this report are confined to forages collected in September, very late in the growing season of the Nome, Alaska vicinity. Therefore if all the forages, with the possible exception of the lichens, had been gathered in the spring and early summer when they were most nutritous (Klein 1970a, b), it is anticipated that the digestibilities of these vascular plants would be higher than recorded herein. Further, if nitrogen is the limiting factor in *in vitro* digestibilities, the discrepancy between *in vitro* and nylon bag results should diminish in the spring and early summer shrubs.

LITERATURE CITED

Ahmadjian, V. 1967. *The Lichen Symbiosis.* Blaisdell Publishing Co., Waltham, Mass. 152pp.

Aksenova, M. J. 1937. [The problem of reindeer feeding.] Arctic Inst. USSR, Sov. Reindeer Indus. 10:5-124. (English summary.)

Banfield, A. W. F. 1954. Preliminary investigation of the barren-ground caribou. Canadian Wildlife Management Bulletin, Series 1. No. 10B. 112pp.

Burkholder, P. R., and A. W. Evans. 1945. Further studies on the antibiotic activity of lichens. Bull. Torrey Botan. Club 72(2):157-164.

Burkholder, P. R., A. W. Evans, I. McVeigh, and H. K. Thornton. 1944. Antibiotic activities of lichens. Proc. Nat. Acad. Sci. U.S.A. 30(9):250-255.

Bustinza, F. 1951. Antibacterial substances from lichens. Endeavor 10(4):95-99.

Courtright, A. M. 1959. Range management and the genus Rangifer: A review of selected literature. MS Thesis. Univ of Alaska. 172pp.

den Braver, E. J. 1974. Den organiska substansens smältbarhet och nedbrytningshastighet i några renbetesvämd med VOS metoden. (Ms).

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. The Am. Naturalist 108(961):269-289.

Hale, M. E. Jr. 1961. Lichen Handbook: A Guide to the Lichens of Eastern North America. Smithsonian Institution Press, Washington, D.C. 178pp.

Hale, M. E. Jr. 1967. The Biology of Lichens. Edward Arnold (Publishers) Ltd., London. 176pp.

Igoshina, K. N. 1937. [The pasture foods and food seasons of the reindeer husbandry of the Pri-Ural district.] Arctic Inst. USSR, Sov. Reindeer Indus. 10:125-195. (English summary.)

Kelsall, J. P. 1968. The Caribou. Queen's Printer, Ottawa, Canada. 340pp.

Kennedy, G. H., and H. W. Titus. ca. 1932. A report on digestibility in reindeer: The digestibility and nutritive properties of a mixed ration of lichen and oats, of a ration of lichens alone, and of a ration of alfalfa hay alone. Typed (carbon) Ms. 54pp.

Klein, D. R. 1970a. Tundra ranges north of the boreal forest. J. Wildl. Mgmt. 23(1):8-14.

Klein, D. R. 1970b. Food selection by North American deer and their response to over-utilization of preferred plant species. Pages 25-46. In A. Watson [Editor], Animal Populations in Relation to Their Food Resources. British Ecological Society Symp. #10. Blackwell Scientific Publications. Oxford and Edinburgh.

McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43(1):99-109.

Nagy, J. G., H. W. Steinhoff, and G. M. Ward. 1964. Effects of essential oils of sagebrush on deer rumen microbial function. J. Wildl. Mgmt. 28(4):785-790.

Nordfeldt, S., W. Cagell, and M. Nordkvist. 1961. Smältbarhetsförsök med renar Öjebyn 1957-1960. Statens Husdjursförsök Bull. 151. 14pp.

Oh, H. K., M. B. Jones, and W. M. Longhurst. 1968. Comparison of rumen microbial inhibition resulting from various essential oils isolated from relatively unpalatable plant species. Applied Microbiol. 16(1):39-44.

Oh, H. K., T. Sakai, M. B. Jones, and W. M. Longhurst. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial function. Applied Microbiol. 15(4):777-784.

Palmer, L. J. 1944. Food requirements of some Alaskan game mammals. J. Mammal. 25(1):49-54.

Pegau, R. E. 1968. Reindeer range appraisal in Alaska. Ms Thesis. Univ. of Alaska. 130pp.

Pegau, R. E., and G. N. Bos. 1972. Caribou report. Alaska Dept. Fish and Game, Fed-Aid Wildl. Rpt. W-17-3 and 4, Job 3-3R. Juneau, Alaska. 20pp.

Pegau, R. E., G. N. Bos, and K. A. Neiland. 1973. Caribou report. Alaska Dept. Fish and Game, Fed-Aid Wildl. Rpt. W-17-4 and 5, Job 3-3R. Juneau, Alaska 68pp.

Person, S. J., R. G. White, and J. R. Luick. 1975. In vitro digestibility of forages utilized by Rangifer tarandus. Pages 251-256. In J. Luick, P. Lent, R. White, and D. Klein [Editors], Biological Sciences Publ. #18: Proc. First Int. Reindeer/Caribou Symp. Univ. of Alaska, Fairbanks, August 9-11, 1972.

Presthegge, K. 1954. Forsøk med lav til drøvtyggere og sven. Norges Landbrukshøgskoles Foringsforsøk. Beretning nr. 76. 87pp. (English summary.)

Scotter, G. W. 1965. Chemical composition of forage lichens from Northern Saskatchewan as related to use by barren-ground caribou. Can. J. Plant Sci. 45(3):246-250.

Scotter, G. W. 1972. Chemical composition of forage plants from the Reindeer Preserve, Northwest Territories. Arctic 25(1):21-27.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. British Grassland Soc. 18(2):104-111.

Van Keuren, R. W., and W. W. Heinemann. 1962. Study of a nylon bag technique for in vivo estimation of forage digestibility. J. Animal Sci. 21(2):340-345.

Vartia, K. O. 1950. On the antibiotic effects of lichens and lichen substances. Ann. Med. Exp. Biol. Fenn. 28(1):1-82.

CHAPTER III.

DIGESTIBILITY AND CHEMICAL COMPOSITION OF SUMMER FORAGES AT PRUDHOE BAY, ALASKA

INTRODUCTION

The objective of this study was to determine the nutritive value of the summer forage of Rangifer tarandus on the North Slope of the Brooks Range. The site was Prudhoe Bay -- one of the intensive study sites of the IBP-U.S. Tundra Biome program. This area is grazed periodically by caribou and is currently undergoing extensive industrial development.

The estimates of in vitro dry matter disappearance (DMD) would be used in collaborative studies to estimate potential individual and population productivity of caribou at Prudhoe Bay. The fiber composition of the dietary constituents was also determined, using a detergent system of analysis, to see how well this correlated with in vitro DMD. DMD was also estimated by the equations of Van Soest and Moore (1965), Van Soest (1965a) and Michell (1973) which have been used for pasture studies with domestic sheep and cattle.

Since caribou consume such a varied diet, it was necessary to investigate the possibility of summing individual plant species estimates of DMD to give an estimate of the digestibility of the overall diet. For this purpose, esophageal fistulated reindeer were used to collect samples of a mixed diet. With these samples both the plant species composition and the in vitro DMD of the diet could be determined and compared with estimates of in vitro DMD based on the sum of the plant species in the sample.

MATERIALS AND METHODS

Forages investigated, together with the portions used in the study are shown in Table 1. These plants included: 2 species of lichens, 6 species of shrubs, 4 species of grass-like plants (including 3 sedges and 2 grasses) and 7 forbs. Two shrub species and 2 grass-like species were represented by more than one combination of plant parts. With the exception of pelleted Cattle Starter #1[a] (PCS), and the lichens Cladonia alpestris and Cetraria cuculata, the plants were collected in the vicinity of Prudhoe Bay, Alaska. The living thalli of the two lichens were collected in the interior of Alaska, about 25 miles SE of Mt. McKinley National Park. Samples of the vascular plants were hand clipped to approximate the portions utilized by grazing reindeer and caribou.

Rumen Inoculum Donors

Three different means of collecting rumen liquor were employed. Conventional collecting techniques were used with 2 rumen fistulated reindeer that were allowed to graze in the Prudhoe Bay area for approximately one month prior to their use as rumen liquor donors. Occasional supplementation with PCS (0.2 - 1.0 kg/d) was given to maintain body condition during periods when the grazing areas available to the tethered animals were restricted due to experimental protocol or severe insect harassment. Rumen liquor was also obtained from two esophageal fistulated

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[a]Ralston Purina Co., St. Louis, Missouri.

TABLE 1. Anatomical composition of forages used for *in vitro* digestion and fiber analyses.

Forage	Anatomical Composition[a]	Acronym
Standard		
Purina Cattle Starter #1		PCS
Lichens		
Cladonia alpestris	Living thalli	Clal
Cetraria cuculata	Living thalli	Cecu
Shrubs		
Dryas integrifolia	25% flower, 75% leaves	Drin
Dryas integrifolia	80% flower, 20% leaves	Drin 2
Salix arctica	50% flower, 50% leaves	Saar
Salix lanata	90% leaves, 10% other	Sala
Salix ovalifolia	50% leaves, 50% flowers	Saov
Salix pulchra	Unknown	Sapu
Salix pulchra	90% leaves, 2% other	Sapu 5
Salix reticulata	90% leaves, 10% other	Sare
Grass-like plants		
Grasses		
Arctophila fulva	99% leaves, 1% other	Arfu
Dupontia fisheri	90% leaves, 10% seed heads	Dufi 16
Dupontia fisheri	98% seed head, 2% other	Dufi 16-2
Sedges		
Carex aquatilis	95% leaves, 5% seed head	..aaq
Eriophorum angustifolium	50% stem, 50% flower	Eran 9
Eriophorum angustifolium	90% leaves, 10% seed head	Eran 12
Eriophorum angustifolium	80% green leaves, 10% brown leaves, 10% seed head	Eran 3

TABLE 1. Continued.

Forage	Anatomical Composition[a]	Acronym
Forbs		
Artemesia richardsoniana	90% leaves, 10% other	Arri
Braya humilis	Whole plant	Brhu
Oxytropis sp.	Whole plant	Oxsp
Parrya sp.	60% flower, 40% leaves	Pasp
Pedicularis lanata	90% flower, 10% leaves	Pela
Saxifragia oppositofolia	50% flower, 50% leaves	Saop
Sedum rosacea	60% leaves, 40% flower	Sero

[a]The percentages listed are approximate and were determined by visual assessment.

reindeer that had been subjected to the same grazing routine. Rumen contents were collected from the fistula during rumination. Rumen samples were also obtained from 3 immobilized caribou following field rumenotomy. Rumen contents were obtained from the rumen fistulated reindeer and caribou using a hollow plexiglass rod (3.2 cm O.D. x 55 cm long). The contents were transferred to a pre-warmed Thermos bottle and transported to the field laboratory where rumen liquor was separated from the contents by squeezing through several layers of muslin cloth. Fermentation with the substrate was initiated within 30 minutes after collection of the inoculum.

Esophageal Fistula Collections

Forage samples were obtained from esophageal fistulated reindeer during a 10 to 20 minute grazing period. During a collection the esophageal plug was removed. When the reindeer ingested forage, the egesta was extruded through the fistula and was collected in a plastic bag fitted with a liner of nylon mesh (10-12 threads per cm). The nylon mesh served to retain the forage and allowed saliva to strain into the plastic bag. This apparatus was fitted into a canvas bag (30 x 18 cm) which supported the plastic bag and liner and which could be attached to the neck of the reindeer (Gaare et al. 1970).

At the end of the collection period the apparatus was taken from the animal and saliva was expressed from the forage sample. A total of 12 individual esophageal fistula (E/F) samples and 6 combinations of E/F samples were analyzed for digestibility. These combinations consisted of several esophageal fistula samples from a specific vegetation

type combined in equal parts (by weight) to obtain a mean sample which would be representative of that vegetation type. The combinations designated E/FC1 and E/FM1 were composed of the same 5 E/F samples, E/FC2 and E/FM2 of the same 2 E/F samples, E/FC3 and E/FBM of 3 E/F samples each and E/FC4 consisted of a mixture of 2 E/F samples.

Fiber Analyses

Forage samples were oven dried at 40 C (>18 hr), ground through a 20 mesh screen using a Wiley Mill, and analyzed using the techniques of Van Soest (1963a, b) and Van Soest and Wine (1967). Details of these techniques appeared in a handbook by Goering and Van Soest (1970). Table 2 lists the chemical composition of the fractions of forage determined by this technique. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined directly. Hemicellulose was calculated as the difference between NDF and ADF; cellulose was calculated as the difference between ADF and ADL. Percent cell solubles were determined as 100-NDF (Colburn and Evans 1967).

Determination of In Vitro Digestibility

In vitro digestibility was determined by the method of Tilly and Terry (1963). After the initial 48 hr incubation of the substrate in buffered rumen liquor, the entire solution was frozen for at least 48 hrs, transferred to a base laboratory, centrifuged and subjected to the second stage incubation with HCl-pepsin (Chapter I).

TABLE 2. Composition of various fractions of forage.

Forage Fraction	Abbreviation	Chemical Components
Neutral detergent fiber (cell wall contents)	NDF (CWC)	Cellulose, hemicellulose, lignin
Cell solubles (cellular contents)	S	All components except cellulose, hemicellulose and lignin
Acid detergent fiber (lignocellulose)	ADF	Cellulose, lignin
Acid detergent lignin	ADL	Lignin

RESULTS

Table 3 lists the *in vitro* digestibilities of 25 Prudhoe Bay forages. Of these, 13 were investigated using rumen liquor from both reindeer and caribou. Although reindeer inoculum elicited higher dry matter disappearances in 9 of these 13 forages, the only forage that was significantly higher ($P<0.05$) was PCS. On the other hand, the lichen *Cetraria cuculata* and the shrub *Salix arctica* were both digested to a significantly greater extent ($P<0.05$) by caribou than by reindeer. Several reindeer-cairbou comparisons involved rather large differences, however the small sample size prohibited statistical evaluation.

Table 4 lists the results of the *in vitro* digestibilities obtained using inoculum from rumen fistulated and esophageal fistulated reindeer. Inoculum from rumen fistulated animals gave significantly higher ($P<0.05$) digestibilities for *Cetraria cuculata*, whereas inoculum collected from esophageal fistulas yielded higher results ($P<0.05$) for *Salix pulchra*. In the other 11 comparisons, including PCS, lichens, shrubs, grass-like plants and mixed forages obtained from esophageal fistulated reindeer, no significant difference ($P<0.05$) was noted between the two sources of rumen contents.

The mean digestibilities of the three main forage types studied are included in Table 5. Of all groups of plants investigated, the grass-like plants exhibited the highest digestibility (mean 63.8%) followed by the forbs (60.1%) and the shrubs (43.6%). The shrubs and forbs showed more variation (SD = 15.2 and 13.0%, respectively)

TABLE 3. Comparison of the *in vitro* digestibilities of Prudhoe Bay forages by reindeer and caribou.

Forage Type[a]	DMD-Reindeer (%±S.D.)	Number of Animals	DMD-Caribou (%±S.D.)	Number of Animals	Significance of Difference
Standard					
PCS	65.8± 3.3	5	55.9± 6.6	3	P<0.05
Lichens					
Clal	18.3±12.5	5	34.4±19.5	3	NS
Cecu	42.3± 8.9	5	73.5± 3.1	3	P<0.01
Shrubs					
Drin	33.2	1	33.1	1	NS
Drin 2	20.9	1			
Saar	52.6± 4.1	3	71.5	1	P<0.05
Sala	34.1± 4.4	3	34.2	1	NS
Saov	36.5± 2.7	2	35.2	1	NS
Sapu	54.5	1			
Sapu 5	56.2	1	48.1	1	NS
Sare	37.6	1			
Grass-like					
Arfu	72.1	1	55.7	1	NS
Caaq	68.4± 6.1	2	56.3	1	NS
Dufi 16	79.5± 2.2	2			
Dufi 16-2	69.6	1	66.9	1	NS
Eran 9	56.3± 3.3	3			
Eran 12	56.7	1	48.1	1	NS
Eran 3	61.9	1			

TABLE 3. Continued.

Forage Type[a]	DMD-Reindeer (%±S.D.)	Number of Animals	DMD-Caribou (%±S.D.)	Number of Animals	Significance of Difference
Forbs					
Arri	65.7± 3.9	2	61.9	1	NS
Brhu	68.1	1			
Oxsp	69.5	1			
Pasp	67.3	1			
Pela	64.0± 7.8	2			
Saop	33.0± 1.5	2			
Sero	54.1	1			

[a]Forage compositions are listed on Table 1.

TABLE 4. Comparison of the esophageal with rumen fistulation techniques for determining *in vitro* digestibility.

Forage	In Vitro Digestibility: Inoculum from Rumen Fistula ($\bar{x} \pm$ S.D.)	n	Inoculum from Esophageal Fistula ($\bar{x} \pm$ S.D.)	n	Significance of Difference
PCS	61.3 ± 7.7	6	64.7 ± 1.3	2	NS
Clal	22.8 ± 19.0	6	29.1 ± 4.0	2	NS
Cecu	61.0 ± 13.9	6	33.1 ± 4.3	2	.05
Saar 4	60.5 ± 9.6	3	47.9 ± 0.8	2	NS
Sala	35.4 ± 3.0	3	30.1 ± 1.6	2	NS
Sapu	48.1 ± 1.5	2	56.2 ± 0.6	2	.05
Caaq	60.2 ± 5.5	2	72.7 ± 0.5	2	NS
Dufi	78.0 ± 1.8	2	81.1 ± 0.5	2	NS
Dufi 16-2	66.9 ± 1.8	2	69.6 ± 2.3	2	NS
E/FC1	41.4 ± 9.2	2	46.4 ± 0.6	2	NS
E/FC2	51.6 ± 1.6	2	45.3 ± 4.8	2	NS
E/FC3	40.1 ± 2.0	2	42.9 ± 0.9	2	NS
E/FC4	37.4 ± 6.4	2	47.5 ± 0.8	2	NS

TABLE 5. Chemical composition and *in vitro* digestion of common caribou forages at Prudhoe Bay.

Forage Type	DMD(%)	Cell Solubles	NDF	ADF	Cellulose	Hemi-cellulose	Lignin
Standard							
PCS	65.8(a)	53.6	46.4	27.2	24.2	19.2	3.0
PCS	55.9(b)						
Lichens							
Clal	24.3(c)	17.0	83.0	4.6	1.7	78.4	2.9
Cecu	42.3(a)	68.4	31.6	3.7	-0.6	27.8	4.3
Cecu	73.5(b)						
$\bar{x} \pm$ S.D.	49.0±20.8	42.7±36.4	57.3±36.4	4.2±0.6	0.6±1.6	53.1±35.8	3.6±1.0
Shrubs							
Drin 1	33.1	64.6	35.4	33.3	14.2	2.2	19.1
Drin 2	20.9(a)	53.8	46.2	48.1	21.1	-1.9	27.1
Saar	52.6(a)	81.5	18.5	16.4	11.9	2.1	4.5
Saar	71.5(b)						
Sala	34.1	79.4	20.6	17.9	13.1	2.7	2.3
Saov	36.1	68.8	31.2	27.3	18.6	3.9	8.7
Sapu	54.5(a)						

TABLE 5. Continued.

Forage Type	DMD(%)	Cell Solubles	NDF	ADF	Cellulose	Hemi-cellulose	Lignin
Sapu 5	52.1	72.9	27.1	17.4	11.4	9.7	6.0
Sare	37.6(a)						
$\bar{x}$ ± S.D.	43.6±15.2	70.2±10.2	29.8±10.2	26.7±12.4	15.1±3.9	3.1±3.8	11.3±9.7
Grass-like							
Arfu	63.9	32.3	67.7	29.0	27.3	38.8	1.7
Caaq	64.4	41.8	58.2	21.7	18.5	36.5	3.2
Dufi 16	79.5(a)	55.0	45.0	19.6	15.9	25.4	3.8
Dufi 16-2	68.2	37.9	62.1	31.0	25.5	31.1	5.5
Eran 9	56.3(a)	42.5	57.5	21.3	16.5	36.2	4.8
Eran 12	52.4	31.5	68.5	24.6	19.4	43.9	5.2
$\bar{x}$ ± S.D.	63.8±8.7	40.2±8.6	59.8±8.6	24.5±4.6	20.5±4.8	35.3±6.4	4.0±1.4
Forbs							
Arri	64.4	57.7	42.3	29.2	24.2	13.1	5.0
Brhu	68.1(a)						
Oxsp	69.5(a)						

TABLE 5. Continued.

Forage Type	DMD(%)	Cell Solubles	NDF	ADF	Cellulose	Hemi-cellulose	Lignin
Pasp	67.3(a)						
Pela	64.0(a)	74.1	25.9	20.9	14.3	5.1	6.5
Saop	33.0(a)	73.1	26.9	35.9	20.9	-9.0	15.4
Sero	54.1(a)						
$\bar{x} \pm$ S.D.	60.1±13.0	68.3±9.2	31.7±9.2	28.7±7.5	19.7±5.0	3.1±11.2	9.0±5.6
Esophageal fistula samples							
E/FC1	43.1	32.6	67.4	35.2	25.4	32.2	9.8
E/FC2	42.8	30.1	69.9	36.2	26.8	33.6	9.5
E/FC3	41.0	37.2	62.8	35.0	23.0	27.8	12.0
E/FC4	40.7	47.5	52.5	32.9	25.6	19.7	7.3
$\bar{x} \pm$ S.D.	41.9±1.2	36.9±7.7	63.2±7.7	34.8±1.4	25.2±1.6	28.3±6.3	9.7±1.9

[a]Reindeer inoculum only.

[b]Caribou inoculum only.

[c]All other DMD's are pooled means using both reindeer and caribou inoculum.

than did the grass-like group (SD = 8.7%). The high standard deviation of the forb group could be attributed to one plant species, Saxifragia oppositofolia. Deletion of this species from the group increased the mean digestiblity from 60.1 to 64.5% and reduced the standard deviation from 13.0 to 5.6%.

Table 5 also lists the mean chemical composition and DMD of the various forages used in this study. Comparative values obtained with reindeer and caribou inoculum are listed separately for PCS, Cetraria cuculata and Salix arctica since significantly different results were obtained for these forages (Table 3).

The differences in the fiber composition of the grass-like plants and the shrubs was striking. The mean NDF composition of the grass-like groups was almost twice that of the shrubs (59.8% vs. 29.8%, respectively), thus the cell solubles concentration in the latter group was higher than in the former. In the grass-like species 59% of the NDF consisted of hemicellulose [mean = 35.3% of total dry matter (D.M.)] whereas hemicellulose made up only 10% of the NDF in the shrubs (3.1% of DM). Although highly variable, the lignin in the shrubs (mean = 11.3% of DM) made up a much larger proportion of the NDF (38%) than in the grass-like forages (mean = 4.0% of DM, 7% of NDF). Cellulose content was more variable than the other parameters, and on the average was higher in the grass-like species (mean = 20.5%) than in the shrubs (mean = 15.1%). Cell solubles, the most readily available component of the plant, were notably higher than the dry matter disappearance in all of the shrubs, whereas the reverse was noted in all of the grass-like species.

The two E/F mixtures collected from the Eriophorum/Salix range (E/FC1, E/FC2) contain a very similar distribution of all paramters measured. The two mixtures from the Dryas dry meadow complex (E/FC3, E/FC4), however, in spite of their similar digestibilities, appear to be structured differently. E/FC3 had a considerably higher NDF content than did E/FC4, 8.1% of which could be attributed to hemicellulose and 4.7% of which resulted from a higher lignin content. The higher NDF, with the resultant lower cell solubles, however, seemed to have no effect on in vitro digestibility.

As shown in Table 6, the mean digestibilities of all E/F samples representing each of the 4 main vegetation types are surprisingly similar, with the Carex lake bed sample being the highest (49.9%), followed by the Eriophorum/Salix association (45.4%), the Dryas dry meadow (44.6%) and the Dupontia brook meadow (44.4%). For comparison with the in vitro DMD, the DMD for each E/F sample was also predicted by summing the products of the mean in vitro DMD for each forage type and the percentage occurrence of that plant type found in the E/F sample (Table 6). The mean DMD for shrubs, grass-like plants, forbs and lichens were obtained from Table 5, and the DMD of the "dead and litter" category was assumed to be 25%. The percentage occurrence of the various plant types in the E/F samples were previously reported (White et al. 1975).

Simple regression equations were computed to ascertain the relationships between digestibility and the fiber composition of arctic forages. Comparisons were also made between the data in this report and the predicted values obtained using previously reported relations

TABLE 6. Digestibility of esophageal fistula samples.

E/F Sample	Number of Observations	Observed Mean DMD (%±S.D.)	Predicted DMD
Eriophorum/Salix association			
1	1	46.0	49.0
2	1	49.9	45.7
3	1	50.9	51.4
M1	1	47.9	48.4
M2	1	46.9	46.2
C1	3	43.1±7.1	48.4
C2	3	42.8±2.4	46.2
	Grand Mean	45.4±4.6	47.9
Dryas type dry meadow			
8	1	37.5	49.5
9	1	56.7	45.4
10	1	46.7	45.8
11	1	50.9	47.9
12	1	53.9	43.3
C3	3	41.0±2.1	46.3
C4	3	40.7±7.4	46.7
	Grand Mean	44.6±7.3	46.4
Dupontia type brook meadow			
14	1	39.3	55.7
15	1	49.6	58.7
16	1	44.4	55.4
BM	1	44.1	56.6
	Grand Mean	44.4±4.2	56.6
Carex type lake bed			
13	2	49.9±1.2	57.2

from Michell (1973), Van Soest (1965a) and Van Soest and Moore (1965). Table 7 lists the pertinent correlations. These data show that NDF does not appear to be closely related to digestibility when compared with the entire range of forages examined in this study, but it is related to DMD in the shrubs and, possibly also in the grass-like plants, although the sample size in the latter was too small to permit a statistical evaluation. Figure 1 shows that although the slopes between digestibility and NDF may be similar for the shrubs and grass-like plants, the intercepts are considerably different.

Significantly correlations were obtained in relationships between both lignin/ADF and lignin/cellulose on DMD, but the correlation coefficients (-0.73, -0.75, respectively) indicate these relationships are of limited value for prediction purposes. A simple regression of lignin on DMD also produced a significant relationship, however Figure 2 shows that the lignin-DMD association appears to be bimodal. Lignin concentrations less than 9% DM do not appear to significantly affect digestibility, however at lignin concentrations greater than this level, there is an inverse linear correlation between lignin and DMD. Multiple regression analyses, using a combination of up to 12 of the parameters, and combinations of the parameters listed in Table 2 did not significantly reduce the variance obtained with simple regression analysis.

Predicted digestibilities using Michell's (1973) equations relating DMD to both NDF and ADF bore essentially no relationship to the experimentally determined DMD as indicated by the correlation coefficients of 0.19 and 0.03, respectively (Table 7). DMD estimated

TABLE 7. Correlations between *in vitro* DMD (y) and several fiber components of forage and comparisons with other predictive equations.

Plant Type	X =	Regression Equation	Correlation Coefficient	Level of Significance
All species	NDF	y = 53.4 - 0.07X	-0.08	NS
Shrubs	NDF	y = 78.7 - 1.27X	-0.77	.05
Grass-like	NDF	y = 113.3 - 0.82X	-0.74	NS
All species	Lignin	y = 61.8 - 1.59X	-0.60	.01
All species<9% lig.	Lignin	y = 61.5 - 1.23X	-0.14	NS
All species (except Cecu and Saar	Lignin/ADF	y = 69.8 - 73.94X	-0.73	.01
All species	Lignin/cellulose	y = 65.6 - 27.56X	-0.75	.01
All species	DMD from (a) Michell (1973)	y = 10.0 + 0.54X	0.19	NS
All species	DMD from (b) Michell (1973)	y = 46.1 + 0.05X	0.03	NS
All species	DMD from (c) Van Soest (1965b)	y = 20.8 + 0.52X	0.53	.01
All species	DMD from (d) Van Soest and Moore (1965)	y = -36.0 + 1.09X	0.65	.01

(a) DMD = 88.3 - 0.3 NDF.

(b) DMD = 88.4 - 0.66 ADF.

(c) DMD = 0.98 (100 - NDF) + NDF (147.3 - 78.9 log [(ADL/ADF)100]) - 12.9.

(d) DMD = 78.2 (1 - ADL/Cell Solubles) + 12.7.

FIGURE 1. The relationship between dry matter disappearance and NDF.

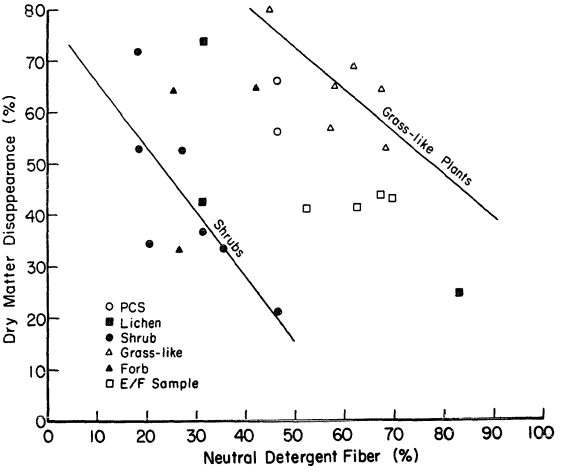
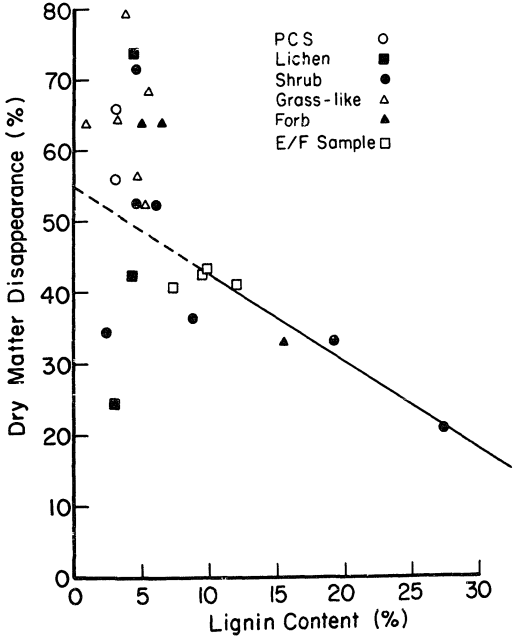


FIGURE 2. The relationship between dry matter disappearance and lignin concentration.



from the prediction equations of Van Soest (1965b) and Van Soest and Moore (1965) relate significantly to the present data (Figures 3 and 4), but both relationships have high residual standard deviations (13.6 and 11.9, respectively) which suggests that these equations may not be suited for predicting digestibility of these forages by reindeer and caribou.

DISCUSSION

Recently, Grant et al. (1974) reported that the diets consumed by inoculum donors appear to have a greater influence on in vitro digestibility than does the species of donor. With the exception of 3 forages tested, our data comparing reindeer and caribou, conspecific animals that have been genetically isolated for many generations, support Grant's hypothesis. The lichen Cetraria cuculata and the shrub Salix arctica were both more highly digested by caribou inoculum, but they have no apparent chemical or anatomical features that would explain these different results. However, lichens were virtually absent from the restricted range utilized by the reindeer. Thus, their rumen microflora may not have been adapted to lichen fermentation. A similar argument can be made for the higher digestibility of PCS pellets with reindeer inoculum, which may be due to the previous adaptation of these deer to a PCS diet. In addition, the experimental reindeer were given supplemental PCS pellets while grazing at Prudhoe Bay.

FIGURE 3. The relationship between experimentally determined DMD and DMD calculated using the predictive equation of Van Soest and Moore (1965).

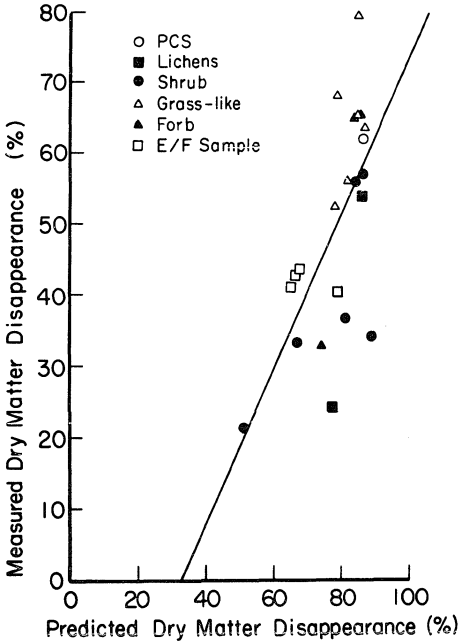
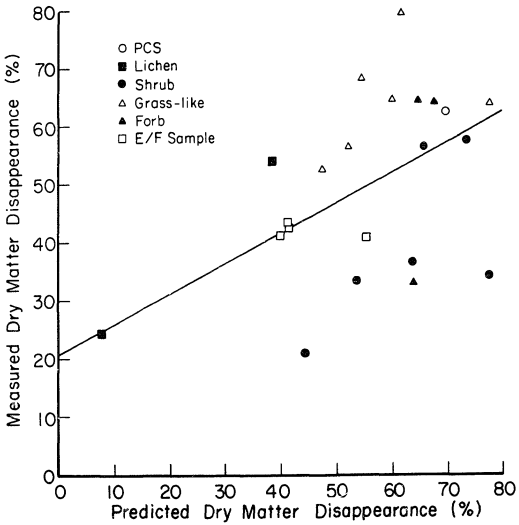


FIGURE 4. The relationship between experimentally determined DMD and DMD calculated using the predictive equation of Van Soest (1965b).



With these exceptions in mind, it appears that digestibilities based on inoculum from the experimental reindeer can be applied directly to caribou nutrition studies, provided the animals are grazed on the same or similar ranges as the caribou. Further, the verification that inoculum obtained from an esophageal fistulated reindeer is equivalent to that obtained from a rumen fistula means that EF animals can serve the dual function of collecting forage samples and providing rumen inoculum. This is an important consideration when working in remote areas, as it means fewer animals must be transported and maintained in sometimes difficult field situations.

Cowen et al. (1970) discuss the futility of determining the nutritive value of a single forage from the typically mixed diet of a wild ruminant since the value of any one component depends upon its relationship to the other forages. Therefore, in an attempt to achieve a more realistic estimate of the digestibility of the forage actually consumed by reindeer, the in vitro DMD of E/F samples collected from animals grazing a given range type were determined. These results were compared with predicted DMD's based on the composition of the E/F sample and the previously determined in vitro digestibility of the forage types in the sample.

The predicted DMD of the four E/F samples from the Dupontia type brook meadow and the singular Carex type lake bed sample all substantially overestimated the observed DMD of the E/F samples. Unfortunately, these samples were all accidentally oven dried at a temperature between 60 C and 80 C, instead of 40 C. Van Soest

(1964, 1965b) has emphasized that the nonenzymic browning reaction, which artificially increases lignification during oven drying, is directly related both to drying temperature and to the moisture content of the forage. Since the E/F samples had been well mixed with saliva during collection, thereby increasing their moisture content, it appears likely that the accidental exposure to high temperature caused increased lignification, thereby artificially reducing the *in vitro* digestion of these E/F samples, confirming Van Soest's (1964) observations.

On the other hand, the mean DMD's of the E/F samples from the *Eriophorum/Salix* and *Dryas* dry meadow forage types, when considered as a group, were very close to the predicted values. The individual sample variations, however, were large (mean difference between predicted and measured DMD was 6.5 per cent), which emphasizes that several opposing phenomena may be affecting this correlation. Besides artificially induced lignification due to the high moisture content of the E/F samples, el-Shazley *et al.* (1961) found that forages high in starch tend to inhibit cellulose digestion, which could also potentially reduce the actual digestibility of an E/F sample. Conversely, Cowen *et al.* (1970) noted that measured values may be higher than predicted on the basis of summing component digestibilities, due to enhancement of digestibility caused by interrelationships of the various forages in the E/F sample. Further, Theurer (1970) stated that forage in E/F samples tends to be of higher quality (higher protein combined with lower fiber) than hand clippped herbage. Despite these limitations, the present data suggest that the digestibility of a hetereogeneous diet can be determined by

the summation of the digestibility estimates of the individual forage species corrected for their frequency of occurrence in the diet. However, further critical experiments must be performed before this hypothesis can be confirmed.

The relationship between the digestibility and the fiber composition of forages has been well documented. Van Soest (1965a), Van Soest and Moore (1965) and Michell (1973), among others, have determined empirical equations relating digestibility to various fiber components of forage.

With the present data, several statistically significant correlations were obtained between in vitro DMD and fiber composition, even when considering the entire spectrum of arctic plants investigated in this study. However, with the exception of lignin (in concentrations exceeding 9%), none of the correlations are precise enough to be used in the predictive sense. Van Soest (1967) has cautioned that undetermined factors (e.g. silicification or the presence of toxic factors) may affect cell wall digestibility. In Chapter II, the effects of toxic compounds or of nutrient limitation were hypothesized. The present results support those contentions, since it appears that something other than cell solubles and fiber composition affects digestibility. Even the summative equations of Van Soest (1965b) and Van Soest and Moore (1965) yield low correlation coefficients when compared to the measured in vitro digestibilities.

The high negative correlations between lignin and DMD in those plants with greater than 9% lignin, however, indicate that, in high concentrations, lignin is a controlling factor in limiting digestion.

Due to the small sample size, however, this does not necessarily negate the cautions of Oh et al. (1966) and Van Soest (1967) against the use of single chemical components to predict the digestibility of different plant species.

Since it is apparent that the relationship between fiber composition and digestibility of arctic plants is not sufficiently exact to be used in prediction equations, it appears that to determine the nutritive value of these plants for Rangifer, one must use in vitro (or in vivo, if time and resources permit) digestibility techniques. It has also been demonstrated that the digestibility of a cross section of the range can be obtained either through the use of in vitro digestibilities of esophageal fistula samples or through summation of the digestibilities of the various plant species that constitute the diet.

Further work is warranted on the disparity between the amount of cell solubles and the in vitro DMD of shrubs. The in vitro DMD's were surprisingly low, particularly when considered in light of their high preference by Rangifer. This high preference was determined using a modified preference index, obtained using previously published data on the range composition in the Prudhoe Bay study area (White et al. 1975). The initial preference index was determined by dividing the percentage of a particular forage-type found in E/F samples by the percentage of that forage-type found in the range (i.e % chosen/% available). With this index, any value exceeding 1 indicates selection for a forage, and any value less than 1 represents selection against a forage. On this basis, forbs appear to be sought after almost three times more

than shrubs, followed by grass-like plants, with "litter and dead" being strongly selected against. However, a serious criticism of this technique is that the potential range of values indicating selection for a forage is from 1 to infinity, and the range of values representing selection against a forage is only from 0 to 1. A trigenometric conversion of all preference indices to the arctangent could be used to make the two ranges of values equal, i.e. to avoid biasing the results toward selection for a given forage. The tangent of the mean arctan, as opposed to the sum of the arctans could then be calculated to avoid meaningless negative values obtained with tans of numbers in the 90° to 180° range, etc., as well as attainment of false results obtained with numbers above 180° (e.g. tan 45° = 1, tan 235° = 1).

Results obtained using the original and arctangent techniques are compared in Table 8. Eliminating the biases inherent in the simple index system through use of the arctan - tan conversion, demonstrates that shrubs, and not forbs, are the most actively sought, followed closely by grass-like plants and forbs, with litter and dead plant material still being strongly selected against. Further, the converted data indicate that reindeer (and presumably caribou) are dietary generalists, apparently not indicating a strong preference for any of the three main forage types investigated in this study. Lichens were not included in this preference scheme due to their scarcity at Prudhoe Bay, but if available, they would probably rank higher than the other forage types, especially in the winter.

TABLE 8. Forage preference indices for Rangifer tarandus.

Vegetation Type	Shrubs		Grass-like		Forbs		Dead and Litter	
	Standard Preference Index	Arc-tan	Standard Preference Index	Arc-tan	Standard Preference Index	Arc-tan	Standard Preference Index	Arc-tan
Eriophorum/Salix	0.58	30.11	1.33	53.06	0.69	34.61	0.75	36.87
Dryas dry meadow	0.52	27.47	2.26	66.13	0.11	6.28	1.29	52.22
Dupontia brook meadow	1.07	46.94	1.06	46.67	2.11	64.64	0.14	8.13
Carex lake bed	3.84	75.41	0.62	31.76	23.33	87.55	0.11	6.23
Salix ovalifolia sand dunes	3.26	72.95	1.38	54.07	1.47	55.77	0.07	4.00
ΣX	9.27	252.88	6.65	251.69	27.71	248.85	2.36	107.45
tan X		3.25		3.02		2.58		-3.18
$\bar{X}$	1.85	50.58	1.33	50.34	5.54	49.77	0.47	21.49
tan $\bar{X}$		1.22		1.21		1.18		0.39

LITERATURE CITED

Colburn, M. W., and J. L. Evans. 1967. Chemical composition of the cell-wall constituent and acid detergent fiber fractions of forages. J. Dairy Sci. 50(7):1130-1135.

Cowan, R. L., J. S. Jordan, J. L. Grimes, and J. D. Gill. 1970. Comparative nutritive values of forage species. Pages 48-56. In Range and Wildlife Habitat Evaluation -- A Research Symposium. U.S. Dept. Agr., Forest Serv., Misc. Publ. 1147.

el-Shazley, K., B. A. Dehority, and R. R. Johnson. 1961. Effect of starch on the digestion of cellulose in vitro and in vivo by rumen microorganisms. J. Animal Sci. 20(2):268-273.

Gaare, E., T. Skogland, and B. R. Thomson. 1970. [Wild reindeer food habits and behavior, Hardangervidda, Jan.-July 1970.] Report from the grazing project of the Norwegian IBP Committee. Statens viltundersøkelser, Trondheim. 97pp. (English summary.)

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agriculture Handbook #379. Agr. Res. Service. U.S. Dept. Agr., Washington, D.C. 20pp.

Grant, R. J., P. J. Van Soest, and R. E. McDowell. 1974. Influence of rumen fluid source and fermentation time on in vitro true dry matter digestibility. J. Dairy Sci. 57(10):1201-1205.

Michell, P. J. 1973. Relations between fiber and water soluble carbohydrate contents of pasture species and their digestibility and voluntary intake by sheep. Aust. J. Exp. Agr. and Am. Husb. 13(61):165-169.

Oh, H. K., B. R. Baumgardt, and J. M. Scholl. 1966. Evaluation of forages in the laboratory: V. Comparison of chemical analyses, solubility tests, and in vivo fermentation. J. Dairy Sci. 49(7):850-855.

Theurer, C. B. 1970. Determination of botanical and chemical composition of the grazing animal's diet. Pages J1-J17. In Proc. of the Nat. Conf. on Forage Quality Eval. and Utilization. Nebraska Center for Continuing Education, Lincoln, Nebraska.

Van Soest, P. J. 1963a. Use of detergents in the analysis of fibrous feeds. I. Preparation of fiber residues of low nitrogen content. J. Assoc. Official Agric. Chemists 46(5):825-829.

Van Soest, P. J. 1963b. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agric. Chemists 46(5):829-835.

Van Soest, P. J. 1964. Symposium on nutrition and forage and pastures: New chemical procedures for evaluating forages. J. Animal Sci. 23(3):838-845.

Van Soest, P. J. 1965a. Comparison of two different equations for prediction of digestibility from cell contents, cell-wall constituents, and lignin content of acid detergent fiber. J. Dairy Sci. 48(6):815. (Abstract)

Van Soest, P. J. 1965b. Use of detergents in analyses of fibrous feeds. III. Study of effects of heating and drying on yield of fiber and lignin in forages. J. Assoc. Official Agric. Chemists 48(4):785-790.

Van Soest, P. J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Animal Sci. 26(1):119-128.

Van Soest, P. J., and L. A. Moore. 1965. New chemical methods for analysis of forages of the purpose of predicting nutritive value. In Proc. IX Intern. Grassland Congr., São Paulo, Brazil. Paper 424.

Van Soest, P. J., and R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assoc. Official Agric. Chemists. 50(1):50-55.

White, R. G., B. R. Thomson, T. Skogland, S. J. Person, D. E. Russell, D. F. Holleman, and J. R. Luick. 1975. Ecology of caribou at Prudhoe Bay, Alaska. In J. Brown [Editor], The Prudhoe Bay Region: Selected Environmental Reports, U.S. Tundra Biome. In Press.

SUMMARY

The aim of this study was to gain insight into the digestibility of indigenous forages consumed by reindeer and caribou. A standard two-stage in vitro digestibility technique was modified for field use, primarily by introducing a freezing and storage step between the first, or fermentation stage and the second, or pepsin digest stage. Several sources of variability seemed inherent to the technique. Between-replicate variability appeared to be associated primarily with a high amount of extraneous dry matter introduced with the inoculum. This problem was compounded by the resultant difficulties in the final filtering of the "digested" (solublized) material from the remaining dry matter. The most effective means of reducing this variability was through careful preparation of the inoculum -- even at the expense of maintaining an aerobic atmosphere for the rumen microbes.

Between-animal and between-day (within-animal) variation was also experienced, and appeared to be related to the diet of the inoculum donor animal. Although not conclusive, rumen inoculum from animals consuming the more heterogenous diets appeared to be more readily adapted to digesting a variety of forages, thereby reducing variability in results. Between-animal and between-day variation could also be reduced by application of a correction factor, calculated by relating all results to a standard forage, common to all trials. It is important, however, that all experimental animals are equally adapted to this standard forage prior to its use (e.g. commerical pellets would be an

unacceptable standard feed in trials utilizing both domestic reindeer that had been accustomed to the pellets and wild caribou that had not been).

It was demonstrated that besides being able to use the in vitro technique to obtain the digestibility of single plant species, there are at least two ways it can be used to estimate the overall digestibility of the forages consumed in a heterogeneous range: measuring the digestibility of an esophageal fistula sample, or, if the composition of the diet is known, summing the digestibilities of the individual forage species, after correcting for their frequency of occurrence in the diet. Although further critical studies are required before conclusive evidence can be obtained, suggestive data indicate that the sum of the individual digestibilities is equivalent to the overall digestibility. This was demonstrated both with prepared mixtures (MRDF and SRF) and with esophageal fistula samples. Besides their obvious value in collecting forage samples for determination of plant selection and overall digestibility of the diet, it was discovered that esophageal fistulated animals can also be used as a source of rumen inoculum. This is an important consideration in reducing the number of animals required for a remote field study. The data also suggest that nutritional data gathered on reindeer may be directly applicable to caribou -- especially when both animals are on the same or similar ranges.

Comparison of the fiber composition of arctic plants with their digestibility yielded several apparent inconsistancies. Digestibilities

of the grass-like plants were about as expected. On the mean, the more mature species (collected in September) were less digestible than those collected earlier in the growing season (July) and although the in vitro digestibilities averaged some what higher than the nylon bag results, the differences were not large. Further, even though no statistically significant relationship between fiber composition and digestibility was found, at least the in vitro digestibility was higher than the cell solubles in all grass-like species.

The more mature shrubs were also less digestible than those collected earlier in the growing season. In contrast to the grass-like plants, however, the in vitro digestibilities of the shrubs were substantially lower than the nylon bag results. The same disparity between techniques was noted with the lichens. The digestibility of the shrubs was also much lower than anticipated based on their apparent palatibility, ascertained from a newly devised preference index. Most significantly, perhaps, the in vitro dry matter disappearance of all the shrubs was substantially lower than the concentration of cell solubles -- a fraction that is generally almost 100% digestible. Of the fibrous components measured, only high concentrations of lignin (>9%) had any strong direct relationship to digestibility, but with the exception of the shrub Dryas integrifolia and perhaps Salix ovalifolia, none of the shrubs or lichens contained high lignin concentrations. Based on this evidence, therefore it appears obvious that some unknown parameter is controlling the in vitro digestiblity of both the shrubs and the lichens.

Circumstantial evidence points to the presence of toxins in the shrubs, and either toxins or insufficient availability of nitrogen in the lichens, as factors limiting *in vitro* digestibility. Further work is required, however, to isolate the controlling factors.